DISSERTATION ON ROLE OF IMMUNOHISTOCHEMISTRY IN THE EARLY DETECTION OF GASTROINTESTINAL LYMPHOMAS WITH SPECIAL REFERENCE TO LYMPHOPLASMACYTIC INFILTRATE

DISSERTATION SUBMITTED TO STANLEY MEDICAL COLLEGE, CHENNAI FOR MD (PATHOLOGY)

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CERTIFICATE

This is to certify that this dissertation entitled **"Dissertation on Role of Immunohistochemistry in the Early Detection of**

Gastrointestinal Lymphomas with special reference to Lymphoplasmacytic Infiltrate" is the bonafide original work of **Dr.K.R.Mohan,** in partial fulfilment of the requirement for **MD (Branch III) Pathology** examination of the Tamil Nadu Dr.MGR Medical University to be held in March 2007.

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DECLARATION

I, **Dr.K.R.Mohan,** solemnly declare that dissertation titled, **"Dissertation on Role of Immunohistochemistry in the Early Detection of Gastrointestinal Lymphomas with special reference to Lymphoplasmacytic Infiltrate "** is the bonafide work done by me at Govt. Stanley Medical College and Hospital during

the period August 2004 to September 2006 under the expert guidance and supervision of **Prof.A.Sundaram MD, Head of the Department,** Department of Pathology.

The dissertation is submitted to the **Tamil Nadu Dr. MGR Medical University** towards partial fulfilment of requirement for the award of **MD Degree (Branch III) in Pathology.**

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CONTENTS

INTRODUCTION

Extranodal lymphomas account for about 40% of all lymphoid neoplasms and the most common site is the gastrointestinal tract. The majority arise primarily in the gut however a minority of gut lymphomas is a result of secondary dissemination from other nodal or extranodal sites.

In most instances the diagnosis is established by endoscopic biopsies or core biopsies and histological interpretation is often challenging. The

common diagnostic problem in diagnosis of GI lymphoma is the differential diagnosis of reactive lymphoid hyperplasia and lymphoma. Often this requires a series of advanced techniques like immunohistochemical staining, PCR technique and Flow cytometry to identify the monoclonality of the lesion.

An interesting feature is the distribution of lymphomas in the GIT that is paradoxical since relatively few lymphomas arise in the terminal ileum where there is greatest concentration of lymphoid tissue and majority arise in the stomach where there is no lymphoid tissue. So any lesion with lymphocytic infiltration in GIT should be carefully examined and lymphoma should be excluded by one way or the other before diagnosing as benign reactive or inflammatory lesion.

This dissertation deals with the role of immunohistochemical staining with CD3, CD20, CD 45 and Cytokeratin AE1/AE3 markers in the GI lesions which showed dense lymphocytic infiltration and in which the lymphoma should be ruled out.

AIMS AND OBJECTIVES

(1) To analyze the usefulness of the immunocytochemistry in the early detection of lymphomas in gastrointestinal biopsies which contain dense mononuclear and lymphoplasmacytic infiltrate; in which it is difficult to exclude lymphoma with routine haematoxylin and eosin stained sections alone

(2) To detect lymphomas of GIT in the early preinvasive stage

(3) To exclude the existence of lymphoma in the tissues with dense lymphoplasmacytic infiltrate by staining immunohistochemically for CD 3, CD20, CD45 and Cytokeratin AE1/AE3.

(4) To confirm the benign inflammatory lesions mimicking lymphomas in routine haematoxylin & eosin stained sections by their polyclonal pattern of staining with the above said markers.

(5) To quantify the intraepithelial lymphocytes by staining immunohistochemically.

REVIEW OF LITERATURE

Immunohistochemistry or Immunocytochemistry (IHC) is a method for localizing specific antigens in tissues or cells based on antigen-antibody recognition; it seeks to exploit the specificity provided by the binding of the antibody with its antigen at the microscopic level. IHC has a long history, extending more than half a century from 1940 when Coons developed an immunoflourescence technique to detect corresponding antigens in frozen sections (1). However only since the early 1990s has the method found general application in surgical pathology. A series of technical developments in IHC have created sensitive detection systems. Among them is the enzymatic label (Horse radish peroxidase) developed by Avrameas and colleagues (2) which in the presence of a suitable colorogenic substrate systems, allowed visualization of the labeled antibody by orthodox light microscopy. One of the critical issues in the development of immunoperoxidase techniques was related to the need to achieve greater sensitivity from the simplest one step direct conjugate method to multistep detection techniques such as the Peroxidase-Antiperoxidase, avidin-biotin conjugate and Biotin streptavidin methods, together with amplification methods and highly sensitive polymer based labeling systems (3).

The development of hybridoma technique facilitated the development of IHC and the manufacture of abundant highly specific monoclonal antibodies, many of which found early application in staining of tissues. Only

when the IHC became applicable to routinely formalin fixed, paraffin embedded tissue sections did it usher in the “brown revolution”. The critical significance of rendering the IHC technique suitable for routine paraffin sections was illustrated by Taylor and colleagues, who in 1974, showed that it was possible to demonstrate antigens in routinely processed tissue (4). Enzyme digestion was introduced by Huang and colleagues as a pretreatment to IHC staining to unmask some antigens that had been altered by formalin fixation, (5). However the enzyme digestion method, while widely applied did not improve IHC staining of the majority of antigens as reviewed by Leong and colleagues (6). Another drawback of enzyme digestion was that it proved difficult to control the optimal “digestion” conditions for individual tissue sections when stained with different antibodies. These difficulties in standardization provided a powerful incentive for the development of a new technique.

The antigen retrieval (AR) technique was developed by Shi and associates in 1991. In contrast to enzyme digestion the AR technique is a simple method that involves heating routinely processed paraffin sections at high temperatures before IHC staining procedures. An alternative method that did not use heating was developed for celloidin-embedded tissues. The intensity of IHC staining was increased dramatically after AR pretreatment, as demonstrated by various articles. (7)

BASIC PRINCIPLES OF IMMUNOHISTOCHEMICSTRY

The object of all stains is to recognize micro chemically the existence and distribution of substances which we have been made aware of macro chemically. The basic principle of IHC as with any other special staining method is a sharp localization of target components in the cell and tissue based on a significant signal-to-noise ratio. Amplifying the signal while reducing the nonspecific background staining (noise) is the major strategy to achieve a satisfactory and practically useful result.

An antibody is a molecule that has the property of combining specifically with a second molecule, termed the antigen. Antigen-antibody recognition is based on three dimensional structure of protein or antigen, which is a critical issue in the understanding of the effectiveness of IHC as well as the mechanisms of AR. The term epitope corresponds to a cluster of amino acids residues that bind specifically to the paratope of an antibody. An epitope is a functional unit and not structural element of a protein and may be classified as continuous and discontinuous. The former are composed, of a continuum of residues in a polypeptide chain, whereas the latter consist of residues from different parts of a polypeptide chain, brought together by the folding of the protein conformation. (8).

The development of hybridoma technique provided an almost limitless source of highly specific antibodies. Although monoclonal antibodies do not guarantee antigen specificity, since different antigens may share similar or

cross reactive epitopes, the practical specificity reflected by IHC is excellent for most monoclonal antibodies tested. In contrast a poly clonal antibody is infact an antiserum, which contains several different molecular species of antibody having varying affinities and even varying specificities against the different antigens or antigenic determinants As a result polyclonal antibodies may give more nonspecific background staining in slides than the staining obtained using monoclonal antibodies.

Comparison of sensitivity and specificity between polyclonal and monoclonal antibodies indicate that polyclonal antibody may be more sensitive but less specific than monoclonal antibody. The reason may be that polyclonal antibody may recognize several different epitopes on a single antigen whereas a monoclonal antibody recognizes only a single type of epitope. Sophisticated amplification techniques, coupled with use of the AR technique have reduced the practical importance of this distinction. Although the specificity of monoclonal antibody has been questioned regarding cross reactivity with non-target molecules, most commercially available monoclonal antibodies are highly reliable for IHC, but again the ultimate specificity control should be the observation of the expected pattern of staining in control tissue sections, with the corresponding lack of unexpected or inexplicable staining reactions (9).

BLOCKING NON-SPECIFIC BACKGROUND STAINING

There are two aspects to the blocking of background staining of tissues, nonspecific antibody binding and the presence of endogenous enzymes. Non specific antibody binding is generally more of a problem with polyclonal antibody, because multiple unwanted antibodies may exist in antiserum. The greater the optimal working dilution, the smaller the problem. If necessary it is advisable to pre incubate the tissue sections with normal serum from the same species of animal in order to occupy unwanted binding sites before incubation with primary antibody.

If enzymes similar to those used as a tracer are present in the tissue they may react with the substrate used to localize the tracer and give rise to problems in interpretation. Inhibiting the endogenous enzymes activity prior to staining can eliminate false positive reactions produced in this way. Peroxidase and substances giving a pseudo peroxidase reaction are present in normal and neoplastic tissues, e.g. leucocytes and erythrocytes and various methods have been described for the destruction of their activity. Incubation in absolute methanol containing 0.5 percent hydrogen peroxide for 10 minutes at room temperature has been reported to produce an almost complete abolition of endogenous peroxidase activity without affecting the immunoreactivity of antigens (10). There are many types of alkaline phosphatase within the human body and most endogenous alkaline phosphatase activity can be blocked using a 1mM concentration of levamisole

in the final incubating medium. The other commonly used enzyme labels glucose oxidase and bacterial beta-2-galactosidase do not present a problem.

IMMUNOCYTOCHEMICAL METHODS

Traditional Direct Technique

The primary antibody is conjugated directly to the labels. The advantage of the directly labeled antibody is that they are simple to use as they only require only one application of the reagent, followed by appropriate chromogen substrate solution. The disadvantage is that the sensitivity when compared to 2 or 3 stage techniques is low. While the most popular direct conjugates are those which are labeled with Fluorochrome, Horse radish peroxidase and alkaline phosphatase directly labeled antibodies are occasionally used (11).

New Direct Technique: (Enhanced Polymer one-step staining method)

A large number of primary antibody molecules and peroxidase enzymes are attached to a dextran polymer backbone. The advantages of this technique are that it is rapid, especially for frozen sections immunocytochemistry and sensitive enough to demonstrate small amount of antigen.

Indirect Techniques:-

The unconjugated primary antibody is applied, followed by a labeled antibody directed against the first antibody. Horse radish peroxidase labeling

is most commonly used and with appropriate chromogen substrate is a more sensitive technique than the equivalent direct method.

Unlabelled Antibody Enzyme-Complex Techniques (PAP & APAAP)

The original immune enzyme bridge method using enzyme specific antibody became rapidly superseded by the improved technique using a soluble peroxidase-antiperoxidase complex (PAP). These complexes are formed from 3 peroxidase molecules and 2 antiperoxidase antibody molecules and are used as a third layer in the staining method. They are bound to the unconjugated primary antibody e.g. Rabbit anti-human IgG by a second layer of bridging antibody that is usually a swine antirabbit applied in excess so that one of its two identical binding sites binds to primary antibody and the other to the rabbit PAP complex. Alkaline phosphatase antibodies raised in mouse by the same principle can be used to form the alkaline phosphatase-antialkaline phosphatase complexes (APAAP). For unknown reasons this form of amplification with the APAAP is not as successful with the PAP technique as excessive background staining can sometimes be a serious drawback.

New Indirect Technique: (Dextran Polymer Conjugate Two Step Visualization System)

The primary antibody in Enhanced polymer one step method is replaced with a secondary antibody. Available in either as antirabbit or an antimouse format it offers greater sensitivity than the traditional indirect

systems, is less time consuming than the 3 stage Avidin-biotin system and does not react with endogenous biotin.

Avidin-Biotin Techniques

These methods rely on the marked affinity of the glycoprotein avidin for biotin, a low molecular weight vitamin. Avidin is present in egg white and is composed of four subunits which form a tertiary structure possessing biotin binding hydrophobic pockets. The oligosaccharide residues present in egg white avidin and its charged properties are reported as giving it some affinity for some tissue components and the result is non specific binding. Also some tissues such as liver contain large amounts of biotin and this can cause further background problems. A similar molecule streptavidin can be extracted from the culture broth of the bacterium Streptomyces avidini. The lack of oligosaccharide residues and neutral isoelectric point is said to give streptravidin advantages over the chicken egg variant.

Biotin (vit H) is easily conjugated to antibodies and enzyme markers. Up to 150 biotin molecules can be attached to one antibody molecule, often with the aid of spacer arms. By spacing the biotins, the larger glycoprotein avidin has room to bind and maximize its strong affinity for biotin.

Variants of avidin-biotin system include peroxidase and alkaline phosphatase either directly bound to avidin or streptavidin. Alternatively the enzymes are biotinylated and 75% of avidin-binding sites are occupied by the biotinylated label forming the avidin-biotin complex. (12).

Hapten Labeling Technique:-

Bridging techniques using haptens such as dinitirophenol and arsenilic acid have been advocated.(13) In this technique the hapten is linked to the primary antibody and a complex is built up using an anti-hapten antibody and either hapten-labeled enzyme or hapten labeled PAP complex.

Immuno Gold silver staining Technique (IGSS)

The use of colloidal gold as a label for immunocytochemistry was introduced by Faulk and Taylor. It can be used in both direct and indirect methods and has found wide usage in ulltrastructural immunolocalisation. It is not widely used in light microscope IHC even after the advantages of silver development reported by Holgate et al in 1983 (14). In this method the gold particles are enhanced by the addition of metallic silver layers. To produce slow forming metallic silver with a tolerance for natural light, the technique uses silver lactate as the ion supplier and hydroquinone as the reducing agent in a protective colloid of gum Arabic at pH 3.5. The method is generally accepted to be more sensitive than the PAP technique but suffers from the formation of fine silver deposits in the background and can be confusing when trying to identify small amounts of antigen.

Tissue Fixation, Processing and Antigen retrieval techniques:

The ideal fixative for IHC studies should not only be readily available but should also be in widespread use to maximize the range and number of samples available for IHC studies. The fixative should preserve antigen

integrity and should limit extraction, diffusion or displacement of antigen during subsequent processing. Also it should give good preservation of morphologic details after embedment in a support medium.

Common fixatives used in Histopathology are divided into two groups- coagulative fixatives such as ethanol and cross linking fixatives such as formaldehyde. Both types of fixatives can cause changes in the steric configuration of proteins which may mask epitopes and adversely affect binding with antibody. In most surgical pathology laboratories the fixative used is 10% neutral buffered formalin. Subsequent processing usually includes a period in 100% ethanol; these tissues are effectively double fixed in both formalin and ethanol.

Using formalin as standard tissue fixative has revealed the following advantages.

1) There is good preservation of morphology for a variety of tissues even after prolonged fixation.
2) Formalin is an economical chemical much cheaper than most alternatives.
3) Carbohydrate antigens are better preserved (15)
4) There is preservation of many antigens through cross linking of protein in situ, thereby avoiding caching out of proteins that may diffuse in water or alcohol. Many low molecular weight antigens are extracted by non-cross linking fixatives such as alcohol or

methanol based solutions but they are well preserved in tissue by formalin in the form of cross linked derivates (16). Traditionally non cross linking precipitating fixatives are believed to be superior to aldehyde fixation in order to retain immunoreactivity for certain larger proteins such as intermediate filaments and immunoglobins.

Formalin may be regarded as a satisfactory fixative for both morphology and IHC provided that a simple and effective AR technique is available to recover those antigens that are diminished or modified.

Antigen Retrieval

A simple heat induced AR technique is now widely applied in pathology. Successful application of the AR technique for routine IHC staining of formalin fixed tissues has rendered the search for alternative fixatives to replace formalin less urgent.

Heat mediated Ag Retrieval – Commonly employed antigen retrieval methods include microwave owen, pressure cooker, steamer, autoclave and water bath. Among the various solutions citrate buffer at pH 6.0, EDTA at pH 8.00 and Tris EDTA at pH 9.9 or 10.0 are most popular.

Proteolytic Enzyme methods.

Digestion methods with proteolytic enzymes are usually limited to sections taken from formalin fixed paraffin embedded tissues, in order to enable those antigens that are blocked by the cross linking of formalin fixative to be uncovered for binding to the relevant antibody. However not

all antigens are blocked and therefore do not requires this treatment. Enzymes like Trypisn, Pepsin and Protease are used for this purpose.

Mucosa Associated lymphoid Tissue (MALT) and the MALT Lymphoma concept:

The anatomical distribution and structure of lymph nodes are adopted to deal with antigens carried to the node in the afferent lymphatic which drain sites at various distances from the nodes. Permeable mucosal sites such as GIT and the bronchi are vulnerable since they are directly in contact with the external environment and specialized lymphoid tissue has evolved to protect them. This is known as MALT and includes Gut associated lymphoid tissue (GALT), bronchus-associated lymphoid tissue (BALT) and other less well characterized entities related to other mucosae.

Histology and Immunology of MALT:

MALT comprises 4 lymphoid compartments:

1) Organized mucosal lymphoid tissue which when concentrated in the terminal ileum, forms Peyer's patches
2) The Lamina propria
3) Intraepithelial lymphocytes
4) the Mesenteric lymph nodes

PEYER'S Patches:-

Organized lymphoid nodules are distributed throughout the small intestine, appendix and colorectum. In the terminal ileum these nodules are

concentrated and collectively form the Peyer's patches which has become the generic term applied to this compartment of MALT.

Peyer's patches are unencapsulated aggregates of lymphoid cells which bear a certain resemblance to lymph node. Each Peyer's patch nodules consist of B and T cell areas and associated accessory cells. The B cell area comprised a follicle center identical to that present in the reactive lymph node with a dark zone at its serosal aspect containing principally centroblasts and a light zone at its mucosal aspect containing mostly centrocytes.Tingible body macrophages are characteristically present. The follicle center is surrounded by a mantle zone of small B-lymphocytes which is broadest at the mucosal aspect of the follicle; surrounding the mantle zone is broad marginal zone in which most of the cells are small to intermediate sized B cells with moderately abundant cytoplasm and nuclei resembling those of centrocytes. The marginal zone extends towards the mucosal surface and some marginal zone B cells enter the overlying dome epithelium to form clusters of intra epithelial B cells. The follicle associated or dome epithelium is a specialized structure containing microfold (M) cells which are thought to sample the intestinal contents which then interact with the underlying lymphoid tissue (17).

IHC studies of Peyer's patches have shown that the follicle centers are identical to those of lymph nodes. They contain follicular dendritic cell bearing immune complexes containing immunoglobin of all isotypes except

IgD on their surface, while the centrocytes and centroblasts likewise synthesize all immunoglobins except IgD. Follicle centre B cells have a mature B cell phenotype and also express CD10. The mantle zone B cells express surface IgM and IgD in contrast to the IgD negative IgM positive marginal zone cells. IgM positive marginal zone B cells are CD5 and CD10 negative. Lateral to the serosal aspect of the B cell follicle there is a T-cell zone equivalent to the paracortical T-zone of the lymph nodes. The immunophenotype of these T cells is the same as that of paracortical T-zone lymphocytes in lymph nodes (18)

The Lamina Propria:

The Lamina Propria is diffusely infiltrated by plasma cells, macrophages and to a lesser extent, B and T lymphocytes. The plasma cells secrete predominantly IgA also IgM, IgG and IgE in the approximate ratio 20:3:3 (19).

The CD4:CD8 ratio of lamina propria T cells is approximately 4:1 and 50% of these cells express the human mucosal lymphocyte antigen HML-1 (CD 103) (20)

Intraepithelial Lymphocytes:

Intraepithelial lymphocytes are present between the epithelial cells throughout the small and large intestine. They are most concentrated in the small intestine where there are approximately 20 intraepithelial

lymphocytes/100 epithelial cells in the jejunum decreasing to 13/100 in the ileum. (21)

Almost all intraepithelial lymphocytes are CD3 positive, CD4 negative, CD5 positive and express HML-1(20).

Mesenteric Nodes

The basic structure of mesenteric lymph nodes is the same as that of peripheral lymph nodes and in the presence of a breach in the intestinal mucosa these nodes cannot be distinguished from peripheral nodes. In normal circumstances however the mesenteric nodes are distinguished by small rather inactive B-cell follicles which may be surrounded by prominent marginal zone, a poorly developed para cortex and prominent dilated sinuses containing transformed B blasts, most of which are synthesizing Ig A.

The MALT Lymphoma concept

In 1983 Isaacson and Wright noted that just as low grade nodal lymphomas recapitulated the features of normal lymph nodes, certain low grade B-cell lymphomas of GIT exhibited features of MALT. In contrast to the nodal lymphomas, the low grade MALT lymphomas tend to remain localized to their site of origin for long periods, seldom disseminate to the bone marrow and respond favorably to local measures such as surgical excision. These favorable clinical features could be explained by the functional properties of MALT as outlined above, the low frequency of bone marrow involvement, for example reflecting the normal traffic of MALT B

cells which unlike their nodal counterparts do not circulate through the bone marrow and home back to their site of origin. A serious obstacle to this argument is the fact that MALT lymphomas only rarely arise at the site where MALT is most abundant, namely terminal ileum and most commonly arise from the sites normally devoid of MALT including the stomach, salivary gland, lung and thyroid.

Histopathology of MALT Lymphomas:

The neoplastic component of MALT lymphomas consist of the B cells surrounding the follicles and selectively infiltrating epithelium to form the characteristic lymphoepithelial lesions and in many cases differentiated plasma cells. The follicles themselves although a characteristic feature of the lymphomas is reactive in nature as are the often considerable population of T-cells. The cytological features of MALT lymphoma cells are not uniform. The most characteristic cells are small to medium sized with a moderate amount of cytoplasm and a nucleus with an irregular outline resembling that of centrocytes. These cells do not conform to any cytological entities recognized by the established lymphoma classifications and because of their resemblance to centrocytes, have been called centrocyte like cells (CCL), (22).

The cytology of MALT lymphoma may vary both between cases and less commonly within a single case. Thus the cells may show a less irregular nuclear outline and bear a closer resemblance to small lymphocytes or alternatively may be characterized by abundant clear staining cytoplasm and

hence resemble monocytoid B cells which are seen in certain reactive lymphadenopathies (23).

Plasma cell differentiation is a feature of most MALT lymphomas and when it occurs in cases in which CCL cells are more lymphocytic in appearance the distinction between MALT lymphoma and immunocytoma becomes difficult and rests on the presence of other MALT features and the site of the tumor

IMMUNOHISTOCHEMISTRY

The distribution of CCL cells in MALT lymphoma corresponds to that of the marginal zone cells in Peyer's patches and there are too striking cytological similarities between CCL cells and marginal zone B cells. The immunophenotype of CCL cells and marginal zone B cells is also very similar (24).

Malignant Lymphoma of the G1T:

The gastrointestinal tract is the commonest site of primary extra nodal lymphoma accounting for between 30 and 50% of cases (25). The lymphomas are almost exclusively of non-Hodgkin's type, primary gastrointestinal Hodgkin's type being extremely rare. There is considerable geographical variation in the incidence of primary gastrointestinal lymphoma, however best illustrated by the extraordinarily high incidence in Middle East.

Definition

Primary nodal lymphoma frequently involves the GIT as a secondary phenomenon; this frequency has almost certainly been underestimated. Strict criteria for a diagnosis of primary gastrointestinal lymphoma are therefore necessary if the incidence is not to be over estimated. The criteria laid down by Dawson et al in 1961, which effectively require that lymphoma be limited to G1T or part there of and its contiguous lymph nodes are still applicable although they do not take account of modern staging procedures which can detect small foci of disease in liver and bone marrow, the presence of which do not necessarily exclude the diagnosis (26).

Sites:

The stomach is by far the commonest site of primary gastrointestinal lymphoma followed by small intestines in the western countries. In the Middle East, however the small intestine is the commonest site and the stomach next in frequency. In both areas colonic, rectal and esophageal lymphoma account for a minority of cases. This distribution of lymphoma in the G1T is paradoxical since relatively few lymphomas arise in the terminal ileum where there is greatest concentration of MALT and the majority arises in stomach where there is no lymphoid tissue.

Staging

The Ann Arbor Staging system for extra nodal lymphomas as modified by Musshoff is most commonly used for staging GI lymphomas. (27)

IE- Lymphoma is confined to the wall of the stomach/intestine

II-1E Involvement of regional lymph nodes those are contiguous with the primary site

II-2E Involvement of regional but non contiguous lymph node group

III Involvement of lymph nodes on both sides of the diaphragm (III-E), Spleen (III-S) or both (III-E+S).

IV There is dissemination to the bone marrow or other non lymphoid organs

Classifications:

Two major classifications of GI lymphomas are as follows:

Primary Gastrointestinal Non-Hodgkin Lymphoma

ISAACSON CLASSIFICATION (28)

B-cell lymphoma

I. MALT-type lymphoma

A. Low grade lymphoma

B.High-grade lymphoma with or without low-grade component

C.Immunoproliferative small intestinal disease

1. Low-grade lymphoma

2. High-grade lymphoma with or without low-grade component

II. Mantle cell (lymphomatous polyposis) lymphoma

III. Burkitt and Burkitt-like lymphoma

IV. Other: nodal equivalent, follicular center cell lymphoma

T-cell lymphoma

I. Enteropathy – associated lymphoma

II. Other types unassociated with enteropathy

III. Rare types

Classification of Gut lymphomas, Updated KIEL (29)

Gastric Lymphomas:

B – Cell Lymphomas	**T – Cell lymphoma**
Low-grade lymphomas	Low-grade lymphomas
Low-grade lymphoma of MALT	Pleomorphic small cell
Immunocytoma	High-grade lymphomas
α – chain disease	Pleomorphic medium and large cell immunoblastic
Centroblastic to Centrocytic	Large cell anaplastic
Centrocytic with MLP	Unclassifiable
High-grade lymphomas	
Centroblastic	
Classic	
Polymorphic	
Centrocytoid	
Multilobated	
Burkitt lymphoma	
Lymphoblastic	
Immunoblastic	
Large cell anaplastic	
Unclassifiable	

The stomach is one of the most common sites of extra nodal lymphomas, with primary lymphoma accounting up to 10% of malignancies at this site and apparently increasing in frequency (30). They occur world wide with equal sex incidence most commonly in the over 50 years age group. It is very important to distinguish between primary gastric lymphomas and nodal lymphomas that are disseminated with secondary involvement of the stomach. A primary gastric lymphoma is defined as a neoplasm where at the time of diagnosis the main bulk of tumor is present in the stomach. Positive mesenteric lymph nodes and bone marrow involvement are regarded as localized metastasis and do not invalidate the diagnosis of primary gastric lymphoma (31).

Although rare examples of Hodgkin's disease (32) and T-Cell lymphoma (33) may be encountered the vast majority of gastric lymphomas are of B-cell origin. The term pseudo lymphoma is obsolete. It is now realized that most of the cases previously described as pseudo lymphoma are, in fact, examples of low grade lymphoma (34). Nevertheless lymphoid hyperplasia may occasionally involve the stomach. On light microscopic appearances alone it may be impossible to separate hyperplasia from low grade lymphoma. It is therefore strongly recommended that to facilitate this distinction immunohistochemical analysis or gene rearrangement studies be performed.

Mucosa-Associated Lymphoid Tumor (MALT)

The majority of primary gastric lymphoma is thought to arise from MALT. These lymphomas have a unique appearance and behaviors (35). They arise in a background of chronic gastritis typically caused by infection with H. Pylori. Low grade and occasionally high grade MALT often responds to antibiotic therapy directed against H pylori and may become reduced in size or regress entirely. Whether the clinical response represents cure or suppression remains unclear at present (35).

MALT lymphomas may be low grade (49%) high grade (34%) or a mixture of low and high grade (17%). The gross appearance of low grade lymphomas may vary-some cases resemble peptic ulcer, others have enlarged mucosal folds but in many instances the mucosa may be flat and either hyperemic or normal. High grade MALTs are typically distinct mass lesions resembling carcinomas.

There are 5 distinct cardinal histologic features characteristic of low grade MALT.

a) an infiltrate of small lymphocytes and small cleaved centrocyte like cells
b) Lymphoid follicles
c) Neoplastic plasma cells
d) Lymphoepithelial lesions
e) Dutcher bodies (PAS-positive intranuclear inclusions)

The CCL cells may have a variety of cytologic appearances. In most instances they are of intermediate size (slightly bigger than a small lymphocyte) with an irregular dense nucleus. The cytoplasm is clear and there is a well defined nuclear membrane. In some cases a few or even many larger cells may be present. These cells resemble centroblasts. In other cases the neoplastic cells may be extremely inconspicuous and show only subtle differences from small lymphocytes. Most MALTs contain a mixture of these cell types. Typically CCL cells are present in perifollicular location where they are thought to arise from marginal zone. As the disease progresses these cells may begin to infiltrate the follicles themselves-follicular colonization. Frequently MALTs cells appear reactive in nature but by specific immunostaining demonstrate light chain restriction (37). In about 1/3rd of MALT, plasma cell differentiation is present particularly in the zone of lamina propria immediately beneath the surface epithelium. Rarely these cells are bizarre and easily recognizable as neoplastic, so that, again demonstration of light chain restriction may be required to prove their neoplastic nature.

Lymphocytes infiltrating the epithelium of the stomach are highly characteristic of MALTs (31) but can also be present in lymphocytic gastritis. To be suggestive of lymphoma, a B cell infiltrate must be present as lymphoepithelial lesion - a discrete cluster of three or more lymphocytes.

These distort or displace adjacent epithelial cells, partially destroying the gland. In lymphocytic gastritis the lymphocytes which are T cells rather than B cells are usually present as single cells within the epithelium.

A characteristic immunophenotype has been detected in MALTs that are constant for tumors with different proportions of cell types. They are usually immunopositive for CD 20, CD21, CD35, and CD79a some cases are also positive for CD 43 and bcl 2. Negative staining results are obtained with CD 5, CD10 and CD23. In particular CD 5 negativity is considered very useful in separating MALTs from other small cell lymphomas (31).

Most high grade lymphomas of the stomach are classified as either diffuse large cell lymphomas or immunoblastic lymphoma in the working classification. On morphologic grounds it is generally not possible to distinguish high grade MALT from diffuse large B cell lymphoma, non MALT type (31). There is good evidence that many high grade lymphomas arise from a pre existent low grade tumor (40). A major diagnostic challenge with biopsy material from this type of case is to separate lymphoma from poorly differentiated carcinoma. Lymphoma cells tend to infiltrate widely in the lamina propria in a sheet like fashion, but they generally spare existing gastric pits and glands. In contrast to low grade lymphoma lymphoepithelial lesions not a common finding in high grade lymphoma. Carcinomas tend to destroy mucosal structures as they infiltrate. The cells are of lymphoma are totally non cohesive with no tendency to form clumps and cords. The nuclei

of large cell lymphomas are vesicular with prominent nucleoli and nuclear membranes.

IHC for CD30, CD 20 and CK may be performed on fixed tissue. Gastric large cell lymphomas are charactertically negative for cytokeratin and positive with CD 30 and CD20 (41)

Other B cell Lymphomas

Other primary gastric lymphomas (non MALT type) include mantle cell lymphoma, follicular lymphoma and Burkitt lymphoma.

Mantle cell lymphomas are multicentric and commonly are seen with polyposis involving multiple sites in G1T. Histologically they contain uniform small or medium sized lymphocytes with round cleaved nuclei and no admixture of blast cells. The immunophenotype is typically positive for CD20, IgM, IgD, CD5, cyclin D1 and CD 43 and negative for CD 23. Burkitt lymphoma and follicular center cell lymphoma of the stomach are rare. Their morphology is identical to that encountered at other sites (42, 43).

Histological scoring of lymphoid infiltrations in the stomach according to Wotherspoon and colleagues: (44)

Score	Diagnosis	Histological features
0	**Normal**	**Scattered plasma cells in Lamina Propria. No lymphoid follicles.**
1	**Chronic active gastritis**	**Small clusters of lymphocytes in Lamina Propria. No lymphoid follicles. No Lymphoepithelial lesions**
2	**Chronic Active gastritis with florid lymphoid**	**Prominent lymphoid follicles with surrounding mantle**

	follicle formation	**zone and plasma cells. No Lymphoepithelial lesions (LEL).**
3.	**Suspicious lymphoid infiltrate probably reactive**	**Lymphoid follicles surrounded by small lymphocytes that infiltrate diffusely in lamina propria and occasionally into epithelium.**
4.	**Suspicious lymphoid infiltrates probably lymphoma.**	**Lymphoid follicles surrounded by marginal zone cells that infiltrate diffusely in lamina propria and in to the epithelium in small groups.**
5.	**MALT lymphoma**	**Presence of dense infiltrate of marginal zone cells in Lamina propria with prominent LEL.**

Morphological and immunohistochemical criteria found useful in the distinction between Gastric MALT lymphoma and gastritis are summarized as follows (45):

1. Marginal zone distribution pattern
2. Lymphoepithelial lesions
3. Follicular colonization
4. Overgrowth of plasma cells in the tips of Lamina propria by ascending CD 20 positive Tumor cells
5. Lack of active gastritis
6. Infiltration of the muscularis mucosa
7. Plasma cell differentiation with Ig light chain restriction. Lymphoepithelial lesions were best recognized after CD 20 staining

which at the same time highlighted lymph follicles allowing decisive assessment of whether or not LEL was follicle associated.

Small and Large Intestines-Lymphoma

Primary malignant lymphomas of the intestinal tract account for approximately 17% of primary small intestinal malignancies and for fewer than 5% of colorectal malignancies. Small intestine lymphomas account for 20-40% of primary gut lymphomas in western populations and they are among the most common malignant tumors of the small intestine. The tumors occur more commonly in men and peak in seventh decade. The presenting features are abdominal pain, weight loss, small intestinal obstruction and acute abdomen. Two thirds are B-cell lesion and one thirds is T-cell lesions (46-48)

Primary colorectal lymphomas are uncommon (49) accounting for only 0.2% of large intestinal malignancies and 10-15% of primary gut lymphomas. The clinical features are no different from those of primary colorectal cancer. Associated conditions are ulcerative colitis and AIDS. Colorectal lymphomas except multiple lymphomatous polyposes are diffuse and polymorphic in appearance, showing mixed populations of centrocyte like, plasmacytoid, centroblast like and immunoblast like cells that are difficult to categorize using standard classifications.

Lymphoma of MALT-Western Type

Most intestinal B-cell lymphomas are of MALT type (50-51) and most of these are of the western type. High grade neoplasms of this type account for 40-60% of all intestinal lymphomas. Many of these high grade lymphomas show evidence of transformation from low grade MALT lymphomas.

High grade lymphomas have sheets of noncleaved cells with round vesicular nuclei and nucleoli. Immunoblasts are present. These lymphomas may also be polymorphic, with large bizarre atypical lymphocytes and admixed mature lymphocytes, plasma cells, histiocytes and eosinophils.

In low grade MALT lymphomas the histologic examination shows infiltrate of CCL cells that often show plasma cell differentiation. LEL are noted but they are less common than in the stomach. Follicular colonization is present and cause confusion with follicular lymphoma. These MALT lymphomas are CD20 positive, CD10 negative, CD5, CD 23, and BCL-1 negative.

MALT-IPSID Mediterranean Type:

This variant which has been classified as IPSID is a disorder commonly manifested by diarrhea, malabsorption, weight loss, abdominal pain and clubbing of digits (52). These manifestations which typically become evident in the second and third decades of life are attributed to diffuse plasma cell infiltrate of the mucosa. In 20-90% of cases the serum contains an

abnormal immunoglobin A molecule devoid of light chains, a condition often called as Alpha-Chain disease (52).

The disease process is usually limited to jejunum and duodenum, however rarely colon may be involved. Grossly the early lesion consists of a diffuse thickening of the small intestinal wall; only when frank lymphoma develops does any gross tumor occur. The early pathologic pattern is a diffuse plasma cell infiltrate of the mucosa without areas of intervening normal mucosa. As the disease progresses the plasma cell infiltrate may invade the bowel wall proper and may involve the regional lymph nodes. Lymphoepithelial lesions and follicular colonization by CCL cells may also be seen (52). IHC studies show that the plasma cells and lymphoma express cytoplasmic and surface Alpha chain.

Enteropathy Associated T-Cell Lymphoma

For many years some patients with malabsorption were noted to develop small intestinal lymphoma. Long term follow up studies of patients with celiac disease reported a 14% incidence of small intestinal lymphoma. Also included in this group were patients with malabsorption that was unresponsive to gluten withdrawal and that was associated with multiple intestinal ulcers (ulcerative jejunitis) (53, 54).These two entities may be considered together because their clinical picture and pathologic features of lymphoma are identical. The clinical manifestation may be malabsorption for years and then the development of lymphoma or patients may present with

abdominal pain at which time multiple small bowel ulcers or lymphoma may be found. The prognosis is extremely poor.

By routine light microscopy a flat sprue like mucosa with areas of malignant histiocyte like cells (medium to large blast like cells with ample cytoplasm and vesicular nuclei with enlarged nucleoli) involving the bowel wall and lymph nodes is seen. Most often the tumor cells are at the base of ulcers, and they may be quite focal. The tumor cells are often obscured by benign inflammatory cells, especially eosinophils. In some cases the lymphoma is so focal that it requires 10 to 20 tissue sections to make a diagnosis of malignancy.

Besides finding pleomorphic malignant histiocytic like cells, one may also see a gradation to mature appearing histiocytes or macrophages, often with erythrophagocytosis. This finding along with that of tumor cells expressing intracytoplasmic α-1 antitrypsin led investigators initially to classify these tumors as malignant histiocytosis. However more sophisticated marker studies indicated that these lymphomas are of T cell origin (53) The malignant cells reacts with the monoclonal antibody HML-1 and have an immunophenotype of CD3, CD7, CD 30 positivity and CD4, CD8, CD22 negativity (55).

Multiple Lymphomatous Polyposis

This distinct clinicopathologic entity is quite uncommon. Its prevalence in large series of GI lymphomas is 5-9 %(56). It primarily affects

male patients with a mean age of 61 years. The lymphomatous process can involve the stomach, small intestine and large intestine. The mean survival is usually less than 3 years.

Grossly the mucosal neoplastic lesions may be nodular, sessile or polypoid. The polyps may produce a confluent studded or cobblestone like appearance or they may be widely spaced with intervening normal mucosa. The polyps range in size from 2 to 3mm to several centimeters. In roughly 50% of cases one also sees a dominant tumor mass that is most often ileocaecal in location. Histologically one observes a nodular or diffuse proliferation of small lymphocytes that produces the polyps. Cytologically, these cells have small, cleaved nuclei and pale cytoplasm; they represent what has been called intermediate lymphocytic lymphoma or mantle zone lymphoma. These cells are of the centrocytic type in the Kiel classification. The early tumor nodules tend to straddle the mucularis mucosa, involving the deep mucosa and sub mucosa with sparing of the superficial mucosa. No epithelial invasion by tumor cells occurs in early lesions. As the lesion progresses focal epithelial invasion and ulceration may develop. Also noted is a pattern of proliferation of neoplastic cells around the germinal centers as has been described in mantle zone lymphoma. Most cases of multiple lymphomatous polyposes are mantle cell lymphoma.

IHC studies have shown that the lesions are B-cell lymphomas that are positive for CD5, Cyclin D1, bcl-1 and negative for CD10, CD23. Malt

lymphomas have also been reported to present as multiple lymphomatous polyposis in the colon. In this situation one sees LEL and IHC markers of lymphoma (MALT) (57).

Multiple lymphomatous polyposis must be differentiated from other lymphoid lesions with multifocal GI involvement namely nodular lymphoid hyperplasia and lymphoid polyposis. In nodular lymphoid hyperplasia one sees benign lymphoid nodules of the small intestine usually in patients with the common variable immunodeficiency syndrome. Multiple lymphoid polyps are benign lesions with well formed germinal centers that are usually seen in children and also in patients with Gardner syndrome. The clinical picture and hitopathologic findings of these two disorders help to differentiate them from multiple lymphomatous polyposes.

Burkitt Lymphoma

Endemic Burkitt lymphoma rarely presents with GI disease however in contrast sporadic Burkitt lymphoma in the western world and the middle East often presents with abdominal pain and obstructive features caused by ileocaecal involvement (47, 50) The tumor cells are small, non cleaved monomorphic medium sized cells with round nucleus and multiple nucleoli and abundant basophilic cytoplasm that may give the cells an cohesive appearance. On touch imprints or smears, cytoplasmic lipid vacuoles are often noted. A classic “Starry sky” pattern is present (55). The tumor cells are immunopositive for CD20, CD10 and negative for CD5 and CD23; Ki 67

staining of more than 95% of cells is helpful in differentiating Burkitt lymphoma from diffuse large cell lymphoma. Most cases have translocation of c-myc from chromosome 8 to the immunoglobulin heavy chain region in chromosome 14 [(t (8, 14)]. (55).

Follicular Center Cell Lymphoma

Follicular center cell lymphoma is uncommon in the GI and it comprises 1% to 3.6% of GI lymphomas (58, 59). These lymphomas occur predominantly in the small intestine, mainly in the duodenum (59), although one study reported ileum as the most common site (58). Endoscopically one sees mucosal nodularity, although at surgery transmural involvement is common. Histologically one can see grades I (46%) II (38.5%) and III (11.5%) using the standard WHO criteria for grading (59). The histopathological pattern is identical to that seen in lymph nodes. By IHC the tumor cells are positive for CD20, CD10, Bcl-6, Bcl-2, and CD79a and negative for CD3, CD5, CD23, and cyclin D1. (59, 60). Endoscopic biopsy materials from grade I lymphoma may be difficult to diagnose and to differentiate from reactive germinal centers. In these instances, immunohistochemistry can be quite helpful in establishing the diagnosis.

Other primary T-Cell Lymphomas

Angiocentric T-cell lymphoma of the intestine is an uncommon disorder. The lesions present as ulcerated or transmurally necrotic small and/or large intestine masses. The tumor cells are large and pleomorphic; they

permeate the bowel wall and they invade blood vessels in an angiocentric and angiodestructive pattern. These tumors are highly aggressive and fatal. The T cells also express Epstein-Barr virus transcripts (61). Many of these lesions may be natural killer cell phenotype, with or without azure granules (seen on Wright stained imprints). Other natural killer like T cell lymphomas of the small intestine with immunoblastic and centrocyte like morphology have been reported (62)

Peripheral T-cell lymphomas unassociated with enteropathy may involve the intestines. Most commonly they involve the small intestine, but colonic disease can also be seen. These lesions tend to be ulcerated; histologically most are high grade lesions. According to the Kiel classification they are pleomorphic medium and large cell, pleomorphic small cell immunoblastic and large cell anaplastic lymphomas. The small cell lesions may show lymphoepithelial lesions. These lymphomas are highly aggressive with high mortality.

Lymphomas Associated with Inflammatory Bowel Disease:

Reticuloendothelial malignant lesions occur in association with inflammatory bowel disease and include lymphomas of both Hodgkin's and non – Hodgkin type and leukemia. Although leukemia with inflammatory bowel disease affects primarily the extra intestinal reticuloendothelial system, lymphomas with both ulcerative colitis and crohn's disease previously were

reported more commonly in the intestinal tract itself. Since the report of NHL of caecum in ulcerative colitis in 1928 there have been 27 complete and 5 incomplete case reports of ulcerative colitis with colonic lymphoma. Colonic lymphoma was documented in 10 patients with crohn's disease.

In the inflamed intestines of patients with ulcerative colitis and crohn's disease, the absolute number of intraepithelial lymphocytes is normal or reduced and the CD4+/ CD8+ ratio is unchanged. However the proportion of cells using γδ T cell receptor may increase. This population is 5% or less in healthy controls but may rise to 30% to 40% in patients with ulcerative colitis. (75)

OESOPHAGUS:

Primary lymphoma of the esophagus is very rare accounting for only 3 cases in a series of 1467 cases reported by Freeman and colleagues. (76).

HODGKIN'S LYMPHOMA:

Hodgkin's lymphoma is extremely rare in GIT. Only 2 cases were found by Lervin et al in a review of 117 lymphomas of the GIT. The large majority of cases so diagnosed in the past are examples of Non-Hodgkin's lymphoma of either B or T cell type (77).

MATERIALS AND METHODS

Source of Data:

A total of 1345 upper gastrointestinal endoscopic/colonoscopic biopsies were received for various diseases in the department of Pathology, Stanley Medical College from Medical and Surgical gastroenterology departments from August 2004 to September 2006. Among these specimens received during the study period only those fifty specimens satisfying the criteria given below were taken up for the study.

Inclusion criteria:

- H/o Chronic diarrhea, abdominal pain
- H/o intestinal obstruction
- Endoscopy – Nodules/small polyps/Thickened Mucosal folds/loss of Mucosal vascularity/Diffuse Thickening of the intestinal wall
- Routine Histopathology :

  i. Dense mononuclear and lymphoplasmacytic infiltration of mucosa, lamina propria with obliteration of glandular structures

  ii.Poorly differentiated carcinoma/ To rule out lymphoma
- CT-Scan – No Hepatosplenomegaly/Para aortic nodes

Exclusion criteria:

HPE – Adenocarcinoma

Method of collection of Data:

The material includes 50 cases of endoscope/colonoscope biopsies from various sites. The clinical details and endoscopic findings were taken from the records in medical records department. 10% buffered formalin has been used for fixation of specimens. The tissues were processed in various grades of alcohol and xylol. Paraffin blocks were prepared subsequently. Multiple thin sections of 5 microns thickness were cut from the paraffin blocks and stained with routine Haematoxylin and Eosin method and also stained immunohistochemically for CD3, CD20, CD45 and Cytokeratin AE1/AE3 (Dako cytomation, USA)

The Haematoxylin and Eosin sections and Immunohistochemically stained sections were studied, quantifications of lymphocytes done and results tabulated. Photomicrographs of the slides are taken.

Quantification:-

In the IHC stained sections the numbers of lymphocytes for each 100 epithelial cells are counted.

OBSERVATION AND RESULTS

Of the 50 biopsies taken for study 30 were from male patients and 20 from female patients. The sites of the biopsies are as follows:-

Stomach - Antrum	6
Stomach - Pylorus	8
Duodenum – II part	9
Duodenum – III part	11
Ileum	1
Caecum	5
Ascending Colon	3
Transverse Colon	4
Rectum	3

In the study majority of the biopsies (20 biopsies) were from the duodenum, 15 from colorectal region, 14 from the stomach and least only one biopsy from the ileum.

The Age distribution of the patients:

Age Group	Number of Patients
0 – 9	1
10 -19	1
20 – 29	6
30 – 39	9
40 – 49	16
50 – 59	11
60 – 69	4

70 – 79	1
80 – 89	1
Total	50

In this study majority of the patients (54%) were in the age group between 40 and 59 years - 16 patients in the age group 40-49 years and 11 patients in 50-59 years. One patients each in the extremes of age like 0-9 years and 80-89 years.

The routine histopathological impressions of these biopsies are as follows:

IMPRESSION	**NO. OF CASES**
STOMACH	
Non specific gastritis with dense lymphoplasmacytic infiltration	**9**
Poorly differentiated carcinoma	**3**
Non specific gastritis To consider lymphoma	**2**
DUODENUM	
Chronic non specific duodenitis with dense	**19**

mononuclear infiltration in the lamina propria	
Tropical sprue	**1**
ILEUM	
Nonspecific ileitis	**1**
COLON	
Non specific colitis	**10**
Ulcerative colitis	**3**
Crohn’s disease	**2**

The iimunohistochemical profile of the biopsies are as follows

CD Marker	**No of cases showing positivity**								
	Non Specific Duodenitis	**Colitis**	**Gastritis**	**Ulcerative colitis**	**Crohn's**	**Ileitis**	**Poorly Diff. Carcinoma**	**Tropical Sprue**	**To consider lymphoma**
3	**3**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
20	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
45	**2**	**4**	**1**	**0**	**1**	**1**	**0**	**1**	**0**
3 & 45	**4**	**1**	**0**	**2**	**0**	**0**	**0**	**0**	**2**
20 & 45	**2**	**2**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
3,20 & 45	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
All Negative	**7**	**2**	**7**	**0**	**1**	**0**	**0**	**0**	**0**
Ck	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**

Quantification of the lymphocytes:-

The numbers of intraepithelial lymphocytes for each 100 epithelial cells are as follows:-

Duodenum:

Less than 20 intraepithelial lymphocytes/100 epithelial cells				**More than 20 intraepithelial lymphocytes/100 epithelial cells**		
No. of cells with				**No. of cells with**		
Sample No.	CD 3+	20+	45+	CD 3+	20+	45+
2	-	-	-	-	20	28
3	-	-	-	-	19	24
5	15	-	-	-	-	-
8	12	18	16	-	-	-
12	18	-	-	-	15	21
14	12	-	-	-	-	-
16	12	-	-	-	-	-
17	-	-	-	10	18	31
20	12	18	-	-	-	-
21	-	-	-	-	26	26
27	-	15	17	-	-	-
34	11	-	12	-	-	-
Total	7	3	2	1	5	5

In the duodenum 7 cases had less than 20 intraepithelial lymphocytes/100 epithelial cells and 5 cases had more than 20 intraepithelial lymphocytes and majority of them were CD 20 positive cells.

One biopsy from duodenum which was reported as Tropical sprue in routine haematoxylin and eosin stained sections showed occasional CD 20 positive cells.

Stomach

Sample No.	No. of Lymphcytes/100 epithelial cells			
	CD3+	20+	45+	CK
10	-	21	21	-
13	-	21	26	-
15	-	36	38	-
26	-	-	-	+
33	-	42	41	-
37	-	-	-	+

The remaining gastric biopsies stained immunohistochemically negative.

Sample Nos. 10 and 13 which were reported as non specific gastritis has 21 and 26 intraepithelial lymphocytes/100 epithelial cells respectively. Sample Numbers 15 and 33 which were reported as gastritis but lymphoma to be ruled out was found to contain 38 and 41 intra epithelial lymphocyltes/100 epithelial cells respectively and majority of them were found to be CD20 cells. These cells were found to infiltrate the muscularis mucosa and were found in clusters within the epithelium.

Samples 26 and 37 which were reported as poorly differentiated carcinoma in which lymphoma to be ruled out stained extensively with cytokeratin.

Ileum

Sample No.	CD3	CD20	CD45
27	-	15	17

In the ileum the intraepithelial lymphocytes amounted to 17/100 epithelial cells

Colon

Sample No.	No. of Lymphcytes/100 epithelial cells		
	CD3	CD 20	CD45
6	18	-	23
7	12	-	19
11	-	-	26
18	-	-	12
22	12	-	12
23	-	15	18
29	-	-	15
31	-	-	12
36	-	12	18
43	-	-	24

44	20	-	-
46	-	15	-

Of the 12 colonic biopsies which stained positively with IHC, 3 cases had less than 13 intraepithelial lymphocytes/100 epithelial cells and 9 cases had more than 13 intraepithelial lymphocytes.

Of the 5 cases reported as inflammatory bowel disease; 4 cases – 3 cases of ulcerative colitis (sample nos-6, 7, 44) and 1 case of crohn's (sample no-11) in the colon stained positively with IHC showed more than 20 intra epithelial lymphocytes/100 epithelial cells and majority of them were CD 20 positive.

DISCUSSION

The gastrointestinal tract is the commonest site of primary extra nodal lymphoma accounting for between 30 and 50% of cases. The lymphomas are almost exclusively of non Hodgkin's type; primary gastro intestinal Hodgkin's disease being extremely rare. The stomach is by far the commonest site of primary gastrointestinal lymphoma followed by small intestine. Colonic, rectal and esophageal lymphoma account for a minority of cases. This distribution of lymphomas in the GIT is paradoxical since relatively few lymphomas arise in the terminal ileum where there is greatest concentration of MALT and the majority arises in the stomach where there is normally no lymphoid tissue.

In the current study over a period of 2 years 50 cases were selected among a total 1345 upper GI and colonoscopy biopsies which were found difficult to exclude lymphoma and differentiate from inflammatory conditions based on the routine haematoxylin and eosin stained sections.

Of the 20 cases taken from the stomach 2 cases were confirmed to be MALT lymphoma (4%) and they are from the pyloric antrum region. Two other biopsies from the stomach both from the antrum of stomach showed lymphoid aggregates and CD 20 positive cells in lamina propria around the lymphoid follicles and also with increased intra epithelial lymphocytes (CD 20+). The remaining 16 gastric biopsies showed no significant immunohistochemically positive cells or only occasional lymphocytes.

The stomach is one of the most common sites of extra nodal lymphomas with primary lymphomas accounting up to 10% of malignancies at this site and occur world wide with equal sex incidence most commonly in the over 50 years age group.

From the above observations, 2 cases of the 4 immunohistochemically positive gastric biopsies were confirmed to be malignant lymphoma of MALT type as they had lymphoepithelial lesions and infiltration of the muscularis mucosa and increased intraepithelial lymphocytes/100 epithelial cells. One of these patients is 33 years of age and the other 55 years of age.

Two other gastric biopsies though lacked the lymphoepithelial lesion they had centrocyte like cells which stained positively for CD 20 in the

lamina propria surrounding the benign appearing lymphoid follicles and also with increased intraepithelial lymphocytes and they were of age 46 and 52 years.

As observed by Franco Cavalli et al, the onset of MALT lymphoma in the stomach where lymphocytes are not normally present is preceded by the acquisition of a mucosa associated lymphoid tissue as a result of H.pylori infection or any other antigenic stimulus (78).

Also another case report by Wolf and Spjut notes that focal lymphoid hyperplasia of the stomach preceded the development of gastric diffuse lymphocytic lymphoma (79). As reported by Scozec et al focal malignant lymphoma supervening in a case of follicular gastritis and focal lymphoid hyperplasia and the possible existence of focal malignant lesion among the lymphoid hyperplasia suggests careful re-evaluation of the specimen and a stringent follow-up of the patients with repeat biopsies (80).

As observed by Cerf-Bensesson N et al intraepithelial lymphocytes are present throughout the small and large intestines where they are approximately 20 lymphocytes/100 epithelial cells, decreasing to 13 lymphocytes as one proceeds from the upper part of the small intestine to the ileum and colon (81).

In this study 7 (14%) cases from duodenum showed less than 20 intraepithelial lymphocytes/100 epithelial cells and 5 (10%) cases showed more than 20 lymphocytes which are significant.

In one case from ileum it is 17 intraepithelial lymphocytes/100 epithelial cells as against 13 cells which could be found normally.

These lymphocytes are found both in groups and in singles and they may be monoclonal which should be confirmed by PCR technology as advocated by Hummel, Oeschger, Barth et al (45)

In this study 12 out of the 15 colonic biopsies stained immunohistochemically positive for lymphocytes, of which 3 cases had less than 13 intraepithelial lymphocytes/100 epithelial cells and 9 cases had more than 13 intraepithelial lymphocytes some in clusters and others in singles where as a normal colon contains only a few lymphocytes.

Emanuale Zucca etal reported (78) that certain parameters are very useful for diagnosing low grade lymphoma in gastric biopsy specimens – prominent lymphoepithelial lesion, moderate cytological atypia of neoplastic lymphocytes and plasma cells with Dutcher bodies. However the absence of these factors does not necessarily exclude the diagnosis of lymphoma. Because lymphoma represents a clonally outgrowth of cells that have acquired certain genetic alterations finding a monoclonal B cell population using the southern blot or PCR technique or immunohistochemically staining for surface immunoglobins might provide the support for the diagnosis.

Depending on these features the patients in this study can be broadly categorized into 3 groups.

Group 1: Those patients who have had their biopsies confirmed to be lymphoma

Group 2: Those cases which have been confirmed to be benign inflammatory lesions.

Group 3: In between these categories lie a significant number of cases which apart from bearing inflammatory changes shows increased intraepithelial lymphocytes than the documented normal quantity and also with borderline changes indicating the co-existence of low grade lymphoma.

Group 1:

In this group fall 2 biopsies from gastric antra (4%) which were interpreted as follicular gastritis and to rule out lymphoma. Later by the IHC study for CD20 and CD 45 confirmed the presence of lymphoma with lymphoepithelial lesion, increased intraepithelial lymphocytes and glandular destruction.

Group II:

Stomach: 10 biopsies from the stomach (20%) showed lymphoplasmacytic infiltrate in the routine hematoxylin and eosin stained sections and which when stained immunohistochemically for CD3, CD 20 and CD 45 showed scanty or no positive reaction indicating their inflammatory nature.

Duodenum: 7 biopsies from the duodenum showed (14%) less than 20 intraepithelial lymphocytes and scant or no CD3/CD20 positive cells which

confirmed the inflammatory nature of these lesions as observed in routine H & E sections and 7 biopsies showed very scant CD 20 cells.

One biopsy from duodenum interpreted as Tropical Sprue has 5-6 CD20 lymphocytes/ 100 epithelial cells.

Rectum and Colon: 3 biopsies from colon (6%) which were interpreted as non specific colitis had few intraepithelial lymphocytes less than 13/100 epithelial cells and they were confirmed to be of inflammatory lesions and 3 biopsies (6%) had scant CD 20 positive cells with 2-3 lymphocytes/ 100 epithelial cells.

Group III:

This group comprises the cases which could not be labeled as lymphoma or neglected as inflammatory pathology based on routine H & E stained sections.

Stomach: 2 cases (4%) from gastric biopsies had lymphoid follicles and more than 20 CD 20 positive intraepithelial lymphocytes and also lymphocytes which are CD20 positive around the lymphoid follicles but the normal stomach usually lacks any lymphoid tissue.

Duodenum: 5 cases (10%) from duodenum had more than 20 intraepithelial lymphocytes/100 epithelial cells and also lymphocytes around glands but duodenum normally has less than 20 intraepithelial lymphocytes/100 epithelial cells.

Ileum: One case from ileum had about 17 intraepithelial lymphocytes/100 epithelial cells.

Colon and Rectum In the colorectal region 9 cases (18%) had more than 15 lymphocytes/100 epithelial cells but normal colon contain only scanty occasional lymphocytes.

Of the 5 colonic biopsies reported as inflammatory bowel disease 4 biopsies (3 of ulcerative colitis and 1 crohn's disease) stained immunohistochemically positive. The ulcerative colitis biopsies showed about 20 intraepithelial lymphocytes/100 epithelial cells and the biopsy interpreted as crohn's disease had 26 intraepithelial lymphocytes/100 epithelial cells whereas in the inflammatory bowel disease the intestinal wall contains reduced or normal intraepithelial lymphocytes which are usually less than 13 lymphocytes/100 epithelial cells in the terminal parts of the intestine (81).

All these cases fall in the borderline area where the inflammatory lesions containing CD 20+ cells may progress to develop malignant lymphoma. A similar picture with focal lymphoid hyperplasia preceding Gastric lymphoma has been reported by Wolf and Spjut (78).

Similarly Scoazec et al (79) reported a case of focal malignant lymphoma supervening in a case of gastric pseudolymphoma in the gastrectomy specimen which was interpreted in endoscopic biopsy as follicular gastritis and pseudolymphoma on different occasions.

These features suggest that the biopsy sections from GIT which could not be neglected as benign inflammatory process because of presence of lymphoid hyperplasia and dense lymphoplasmacytic infiltrate staining truly for CD20, 45, 3 markers must be analyzed for monoclonality using other techniques like PCR and by staining for surface immunoglobins as there are chances for these lymphocytes to undergo genetic alterations in response to antigenic stimulus.

So these patients need rigorous follow up biopsies and other improved modalities like PCR –technique and Immunohistochemical staining of surface Immunoglobins for monoclonal detection so that the disease can be diagnosed in the early stages and treatment initiated.

Summary and Conclusion

During the study period between August 2004 to September 2006 fifty endoscopic/ colonoscopic biopsies with dense lymphoplasmacytic infiltration were taken up to detect lymphomas using Immunohistochemistry.

1. 4% of cases were diagnosed as lymphomas and they are gastric biopsies. These cases have been reported in the routine haematoxylin and eosin stained sections as chronic inflammatory lesions in which lymphoma to be ruled out and this observation emphasizes the importance of marker studies in the early diagnosis of lymphomas which could otherwise be missed out as benign inflammatory condition.

2. Two biopsies from the stomach had increased number of intraepithelial lymphocytes apart from lymphoid follicles which are atypical and plasmacytic requiring further techniques like PCR to confirm the monoclonality of the lymphocytes.
3. Five biopsies from the duodenum (25% of duodenal biopsies) showed more than 20 intraepithelial lymphocytes per 100 epithelial cells and dense CD 20 positive lymphoid tissue as highlighted by the immunohistochemically CD 20 positive cells which remain in the borderline short of designating it lymphoma. These lesions require intense further follow-up with repeat biopsies and other techniques like PCR and other CD and surface immunoglobin markers to confirm the malignant potential of these lesions.
4. Nine biopsies from the colon (60% of colonic biopsies) showed intense CD 20 and CD 3 positive lymphocytes and more than 13 intraepithelial lymphocytes per 100 epithelial cells which are more than the permissible lymphocytic population in this region requiring repeat biopsies and other marker PCR studies.
5. The biopsies which were categorized as Group III in this study had atypical dense CD 20 positive cells amongst the epithelium and between the lymphoid follicles which were in increased numbers than the normal points to their neoplastic nature requiring further

follow up biopsies and other marker, PCR studies to confirm their monoclonality.

Preparation of slides:

Gelatin coated slides are prepared for taking sections to be stained by IHC.

Chrome alum	- 0.05 g
Gelatin	- 0.3 g
Distilled Water	- 100ml

First chrome alum is added to distilled water and then the distilled water is heated to 60C. Gelatin is added slowly to the heated distilled water. Glass slides are then dipped in this solution and dried overnight.

Preparation of Tris Buffered Saline (TBS)

0.005 M TBS

Distilled water – 10 liters

Sodium Chloride – 80 g

TRIS (Hydroxymethyl Methylamine) – 6.05 g

1M HCL - 44ml

Final pH is adjusted to 7.6 with either 1M Hcl or 0.2M Tris solution

Antigen Retrieval

The slides are placed in 0.005 M. Tris buffered saline in the coplin jar and capped. The jar is then heated in a 750 W domestic microwave oven for 15 minutes (5minutes in low power, 5 minutes in medium power and 5 minutes in full power) pausing only to top up the fluid.

Procedure:

1. Dewax the Sections and bring sections to distilled water
2. Antigen retrieval using TBS by Microwave oven heating
3. Bring sections to TBS
4. Drain and wipe off excess TBS around sections
5. Incubate in endogenous peroxidase blocking reagent for 45 minutes
6. Gently wash the slides in TBS
7. Incubate in diluted primary Antibody for 45 minutes
8. Repeat step 6
9. Incubate in diluted biotinylated bridge reagent for 45 minutes
10. Repeat step 6
11. Incubate in Biotin-streptavidin horse-radish peroxidase for 45 minutes
12. Repeat step 6

13. Incubate in DAB substrate solution for 10 minutes
14. Wash in running water, counter stain in haematoxylin, dehydrate, clear and mount

Preparation of slides:

Gelatin coated slides are prepared for taking sections to be stained by IHC.

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Gelatin	- 0.3 g
Distilled Water	- 100ml

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Procedure:

15. Dewax the Sections and bring sections to distilled water
16. Antigen retrieval using TBS by Microwave oven heating
17. Bring sections to TBS
18. Drain and wipe off excess TBS around sections
19. Incubate in endogenous peroxidase blocking reagent for 45 minutes
20. Gently wash the slides in TBS
21. Incubate in diluted primary Antibody for 45 minutes
22. Repeat step 6
23. Incubate in diluted biotinylated bridge reagent for 45 minutes
24. Repeat step 6
25. Incubate in Biotin-streptavidin horse-radish peroxidase for 45 minutes
26. Repeat step 6

27. Incubate in DAB substrate solution for 10 minutes

28. Wash in running water, counter stain in haematoxylin, dehydrate, clear and mount

PROFORMA

Name: Hospital

Age: Diagnosis:

I.P. No. Date of Admission:

Unit:

Address: Date of Discharge:

Complaints:

Diarrhea

Pain abdomen

Malena

Dysphagia

Altered bowel habits

Past History:

H/0 similar complaints in past:

Treatment underwent if any:

H/0. any drug intake

Personal History:

Family History:

H/o. similar disease

H/o. any GI tumors in the family

Examination:

Built:

Nourishment:

Ht:

Wt:

Anemia:

Others:

Pulse:

BP:

P/A

Scar:

Mass:

Size of Mass:

Tenderness:

Consistency:

Borders:

Provisional Diagnosis:

Investigations:

Hb:

RBC:

PCV:

BT & CT:

Blood Urea:

S. Creatinine:

USG/CT: No.

Endoscopy:

Finding:

Biopsy Report:

PROFORMA

Name: Hospital

Age: Diagnosis:

I.P. No. Date of Admission:

Unit:

Address: Date of Discharge:

Complaints:

Diarrhea

Pain abdomen

Malena

Dysphagia

Altered bowel habits

Past History:

H/0 similar complaints in past:

Treatment underwent if any:

H/0. any drug intake

Personal History:

Family History:

H/o. similar disease

H/o. any GI tumors in the family

Examination:

Built:

Nourishment:

Ht:

Wt:

Anemia:

Others:

Pulse:

BP:

P/A

Scar:

Mass:

Size of Mass:

Tenderness:

Consistency:

Borders:

Provisional Diagnosis:

Investigations:

Hb:

RBC:

PCV:

BT & CT:

Blood Urea:

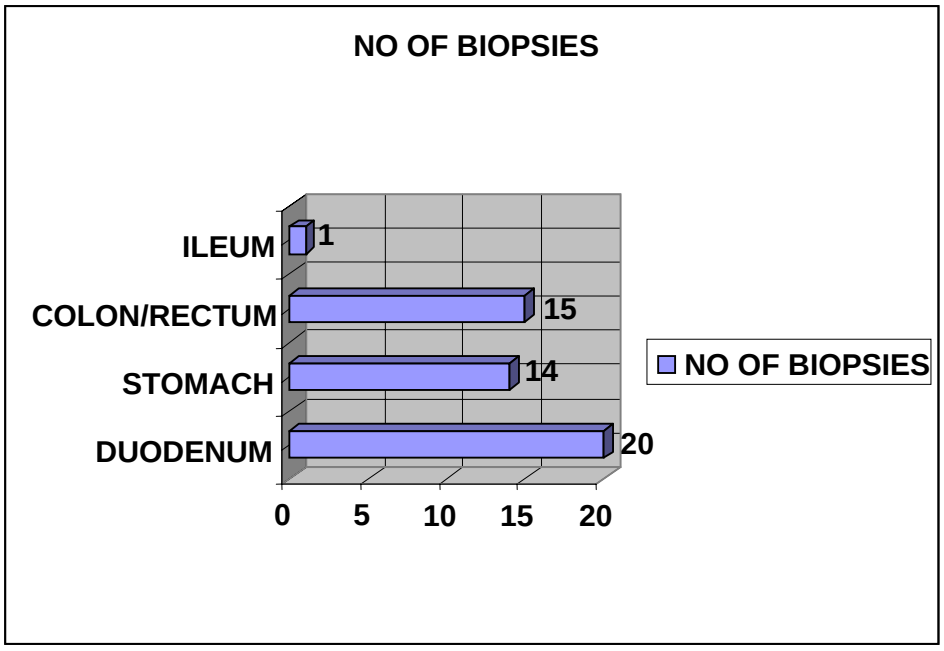
S. Creatinine:

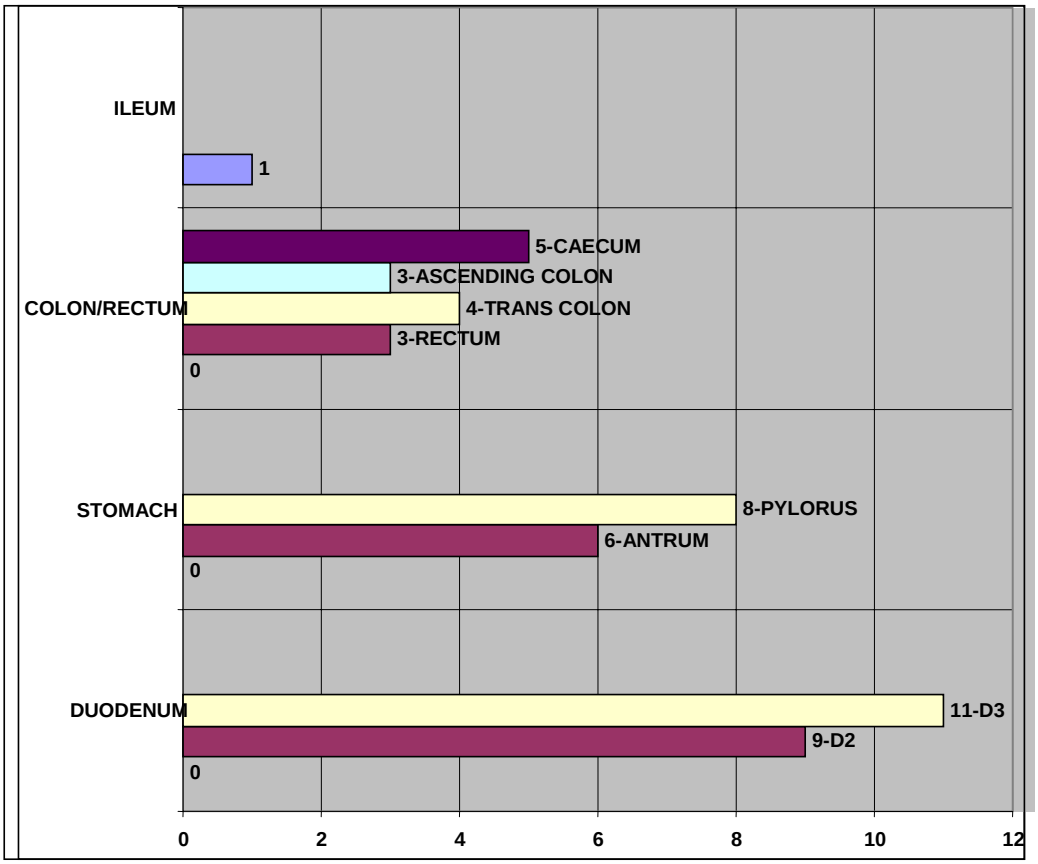
USG/CT: No.

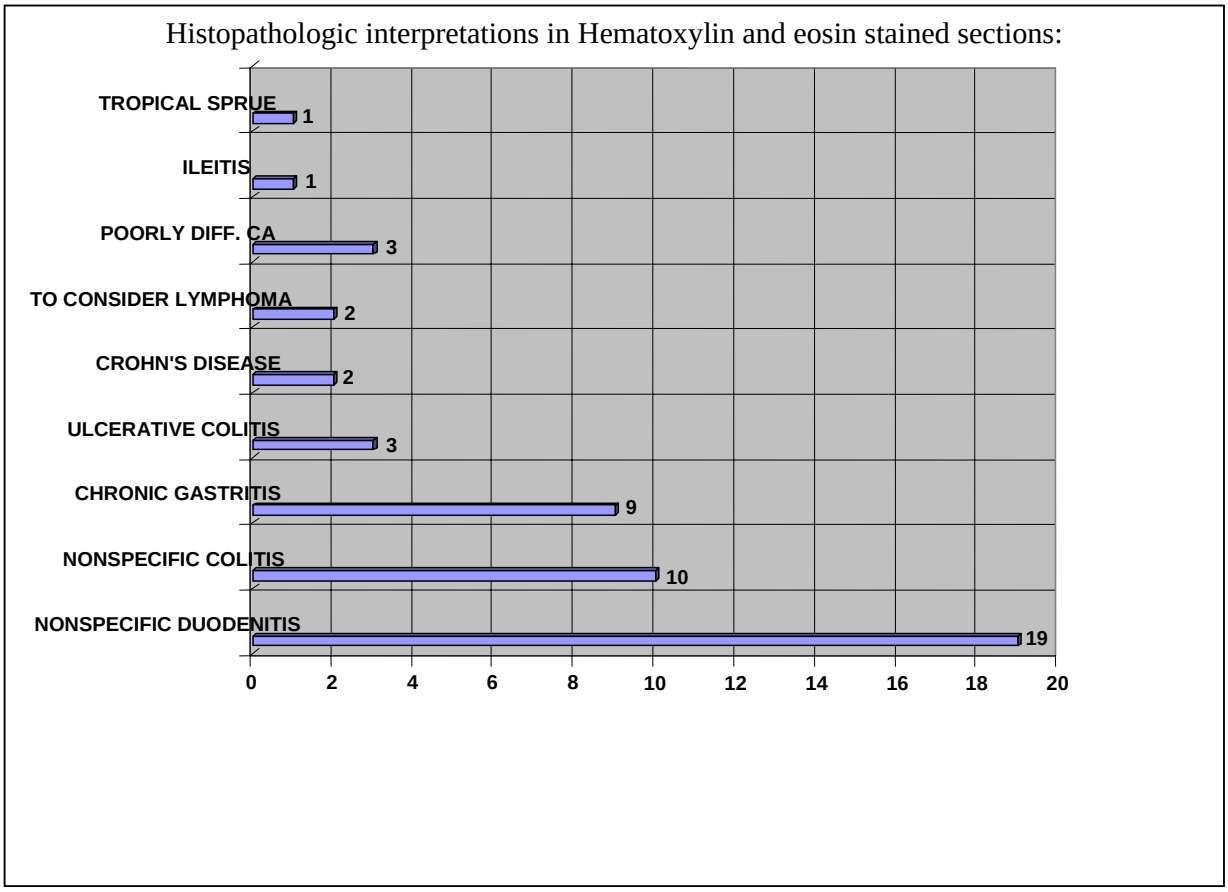
Endoscopy:

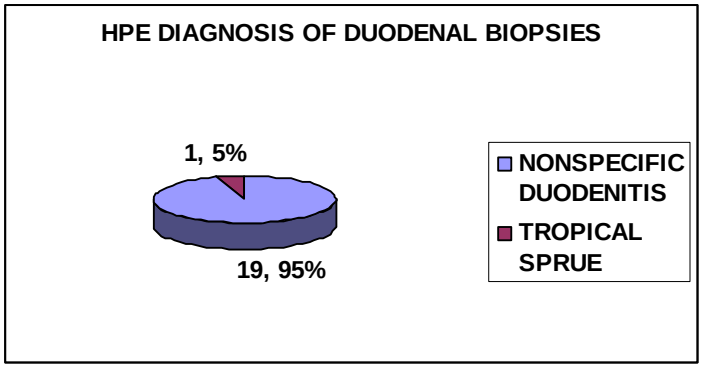
Finding:

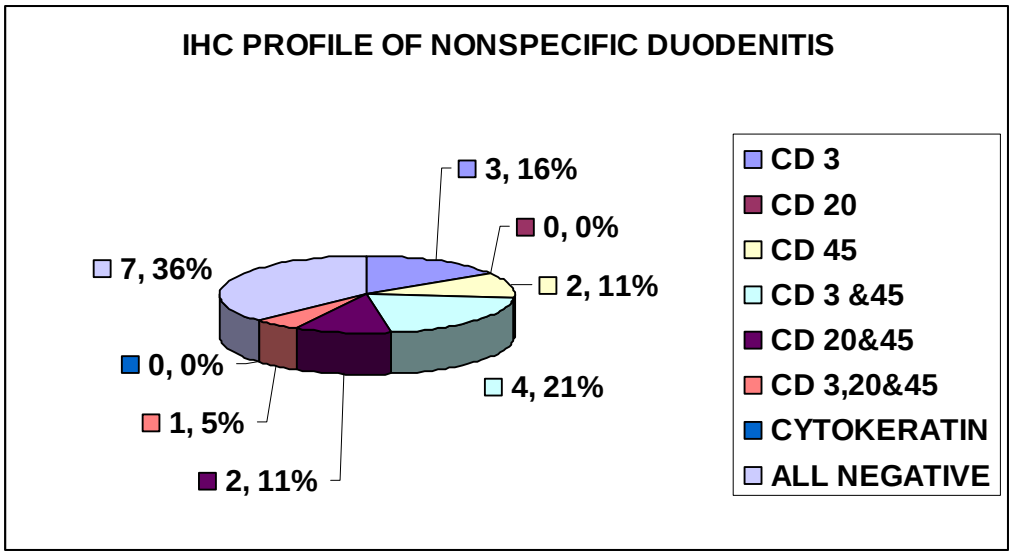
Biopsy Report:

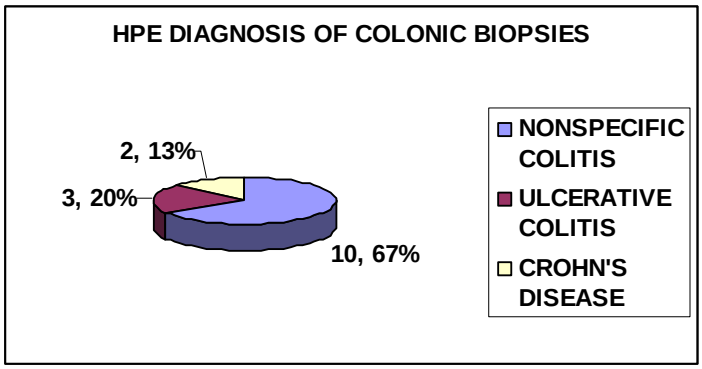


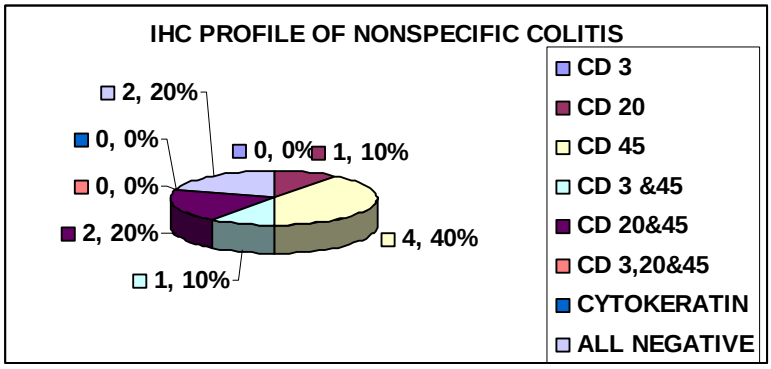

ILEUM
1
COLON/RECTUM
5-CAECUM
3-ASCENDING COLON
4-TRANS COLON
3-RECTUM
0
STOMACH
8-PYLORUS
6-ANTRUM
0
DUODENUM
11-D3
9-D2
0
0
2
4
6
8
10
12

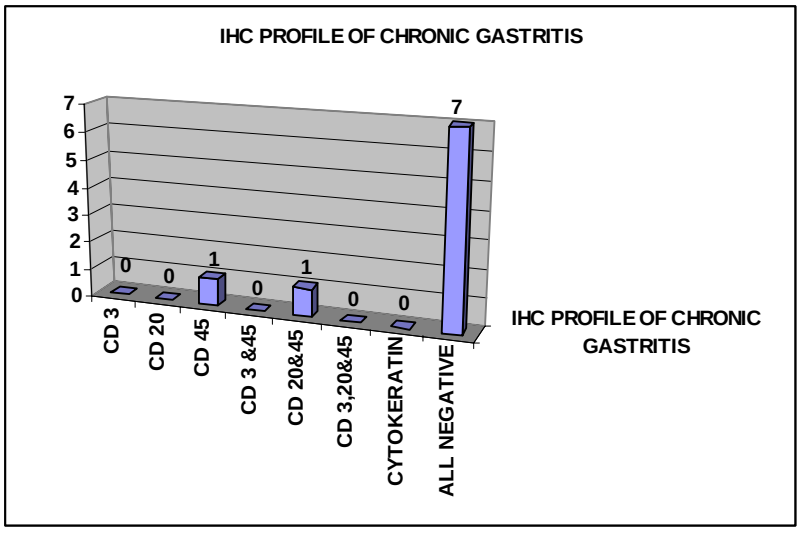

Histopathologic interpretations in Hematoxylin and eosin stained sections:
TROPICAL SPRUE
1
ILEITIS
1
POORLY DIFF. CA
3
TO CONSIDER LYMPHOMA
2
CROHN'S DISEASE
2
ULCERATIVE COLITIS
3
CHRONIC GASTRITIS
9
NONSPECIFIC COLITIS
10
NONSPECIFIC DUODENITIS
19
0
2
4
6
8
10
12
14
16
18
20

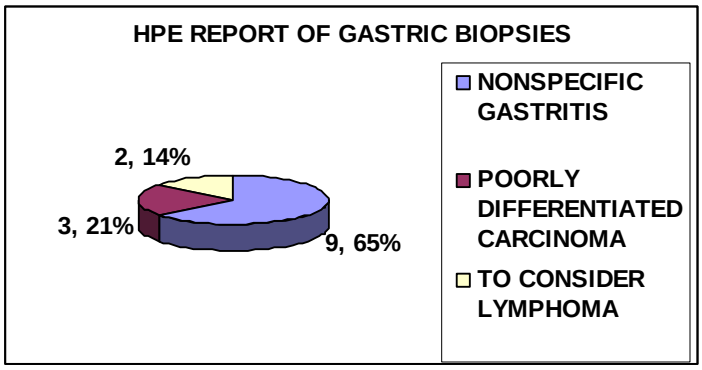

HPE DIAGNOSIS OF DUODENAL BIOPSIES
1, 5%
19, 95%
NONSPECIFIC DUODENITIS
TROPICAL SPRUE

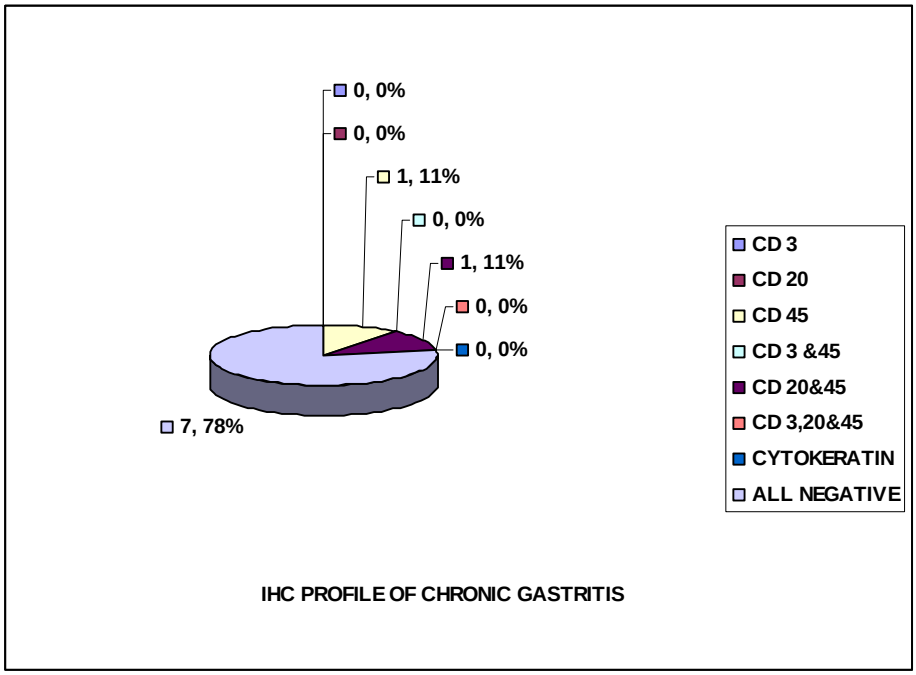

IHC PROFILE OF NONSPECIFIC DUODENITIS
3, 16%
0, 0%
2, 11%
4, 21%
2, 11%
1, 5%
0, 0%
7, 36%
CD 3
CD 20
CD 45
CD 3 &45
CD 20&45
CD 3,20&45
CYTOKERATIN
ALL NEGATIVE

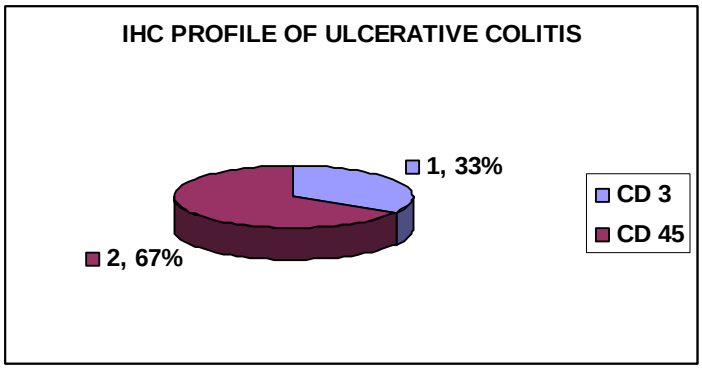

HPE DIAGNOSIS OF COLONIC BIOPSIES
2, 13%
3, 20%
10, 67%
NONSPECIFIC COLITIS
ULCERATIVE COLITIS
CROHN'S DISEASE

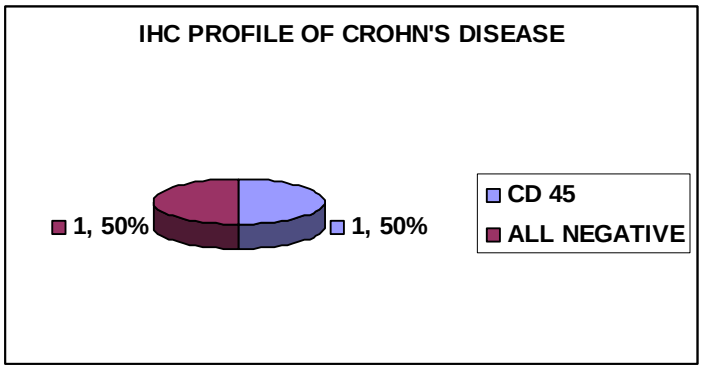

IHC PROFILE OF NONSPECIFIC COLITIS
2, 20%
0, 0%
0, 0%
1, 10%
0, 0%
2, 20%
4, 40%
1, 10%
CD 3
CD 20
CD 45
CD 3 &45
CD 20&45
CD 3,20&45
CYTOKERATIN
ALL NEGATIVE

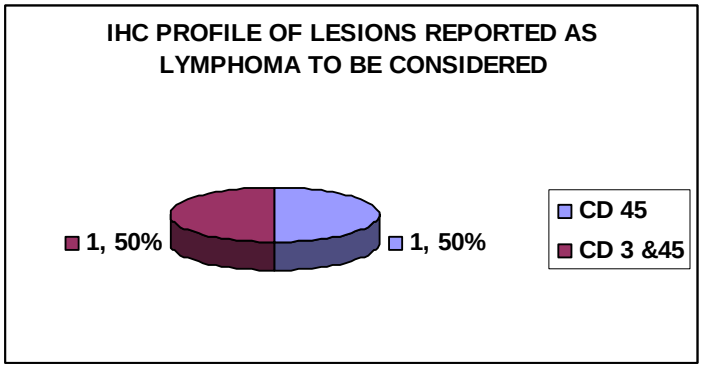

IHC PROFILE OF CHRONIC GASTRITIS
7
6
5
4
3
2
1
0
0
0
1
0
1
0
0
7
CD 3
CD 20
CD 45
CD 3 &45
CD 20&45
CD 3,20&45
CYTOKERATIN
ALL NEGATIVE
IHC PROFILE OF CHRONIC GASTRITIS

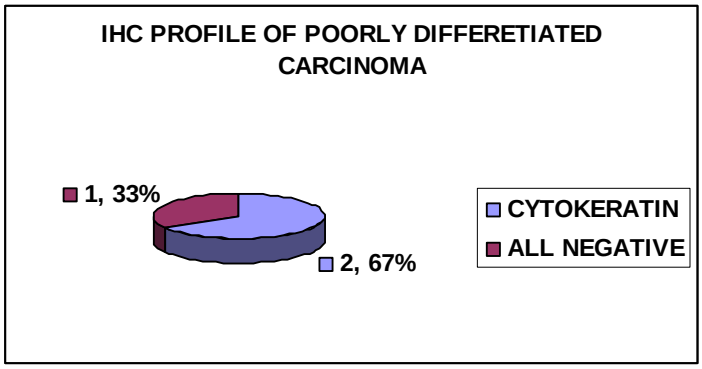

HPE REPORT OF GASTRIC BIOPSIES
2, 14%
3, 21%
9, 65%
NONSPECIFIC GASTRITIS
POORLY DIFFERENTIATED CARCINOMA
TO CONSIDER LYMPHOMA

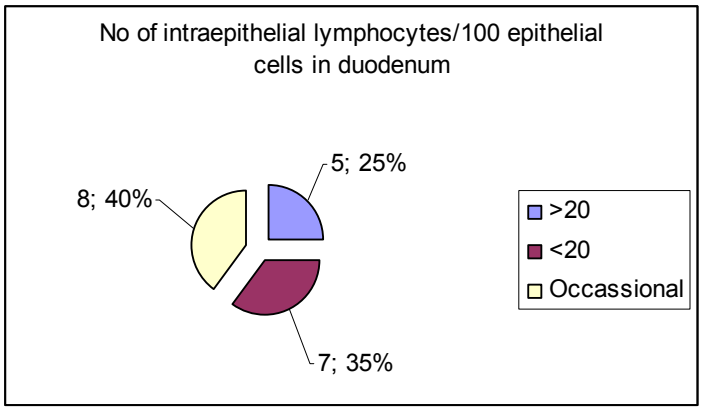

0, 0%
0, 0%
1, 11%
0, 0%
1, 11%
0, 0%
0, 0%
7, 78%
CD 3
CD 20
CD 45
CD 3 &45
CD 20&45
CD 3,20&45
CYTOKERATIN
ALL NEGATIVE
IHC PROFILE OF CHRONIC GASTRITIS

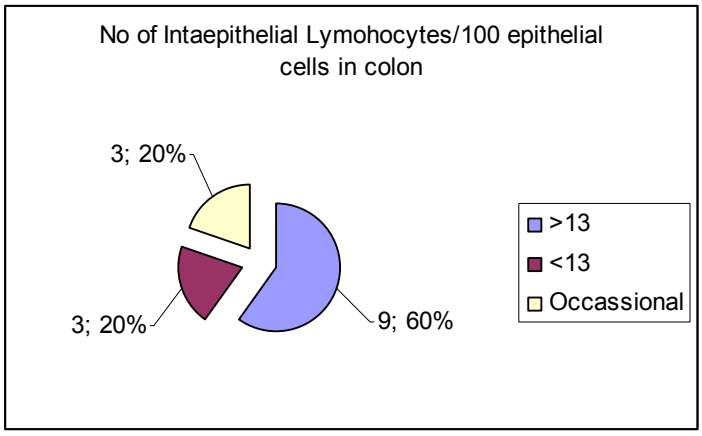

IHC PROFILE OF ULCERATIVE COLITIS
1, 33%
2, 67%
CD 3
CD 45

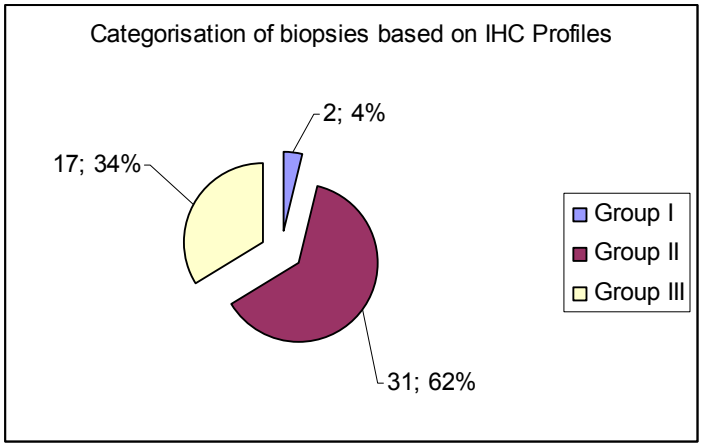

IHC PROFILE OF CROHN'S DISEASE
1, 50%
1, 50%
CD 45
ALL NEGATIVE


IHC PROFILE OF LESIONS REPORTED AS LYMPHOMA TO BE CONSIDERED
1, 50%
1, 50%
CD 45
CD 3 &45


IHC PROFILE OF POORLY DIFFERETIATED CARCINOMA
1, 33%
2, 67%
CYTOKERATIN
ALL NEGATIVE


No of intraepithelial lymphocytes/100 epithelial cells in duodenum
5; 25%
8; 40%
7; 35%
>20
<20
Occassional


No of Intaepithelial Lymohocytes/100 epithelial cells in colon
3; 20%
3; 20%
9; 60%
>13
<13
Occassional


Categorisation of biopsies based on IHC Profiles
2; 4%
17; 34%
31; 62%
Group I
Group II
Group III

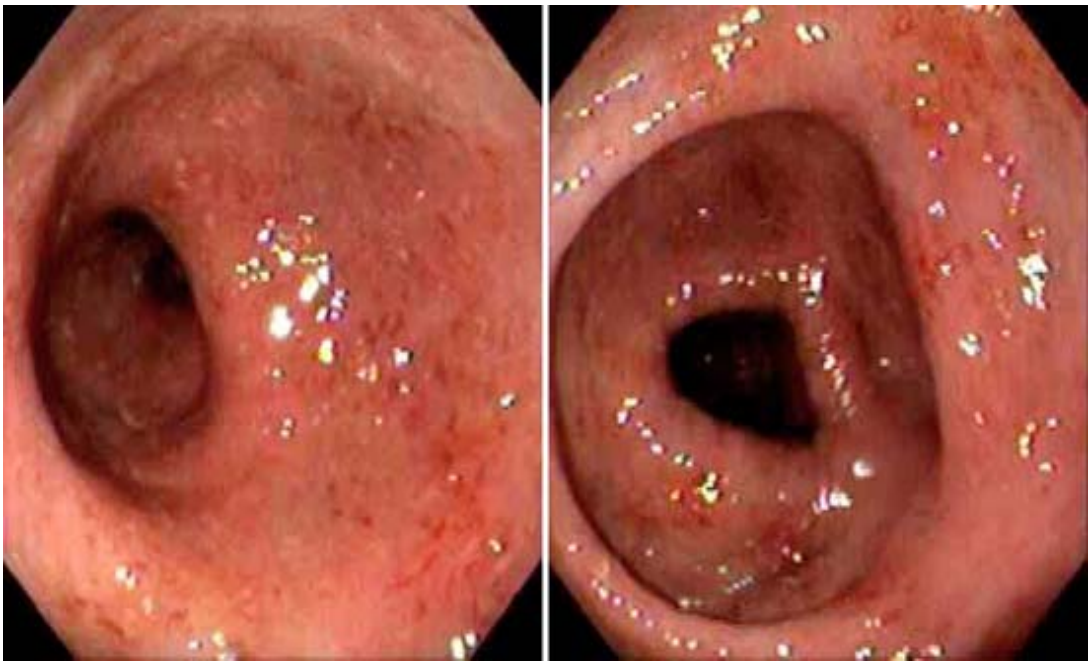


Fig.1: Endoscopic view of the normal intestine.

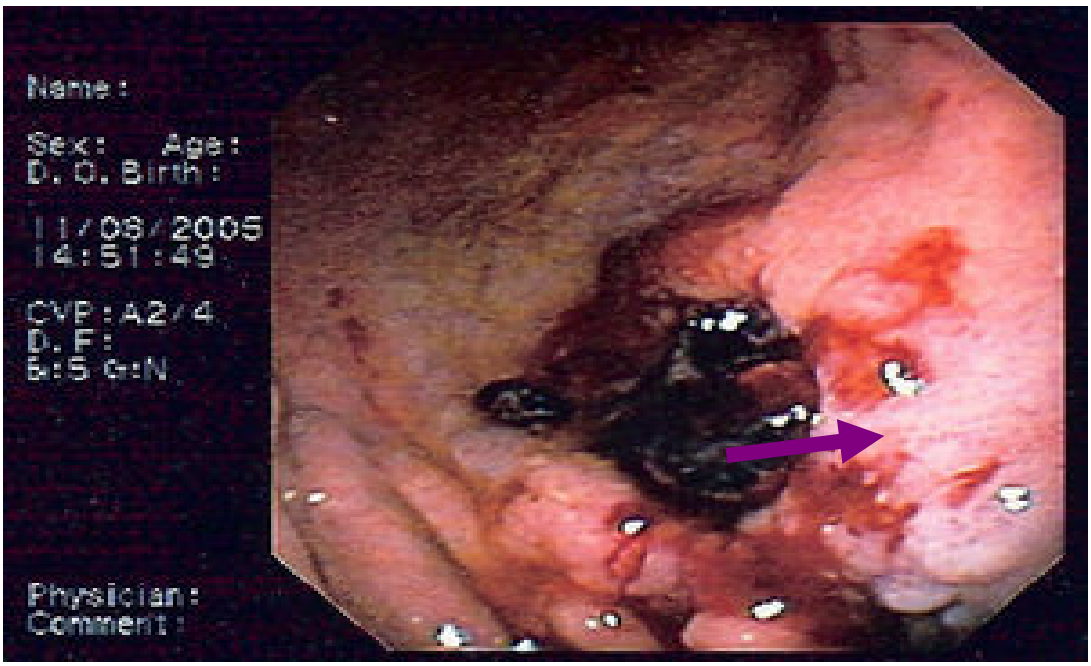


Fig.2: Endoscopy shows nodular lesions in the pyloric antrum region that was then confirmed to be lymphoma using Immunohistochemical staining.

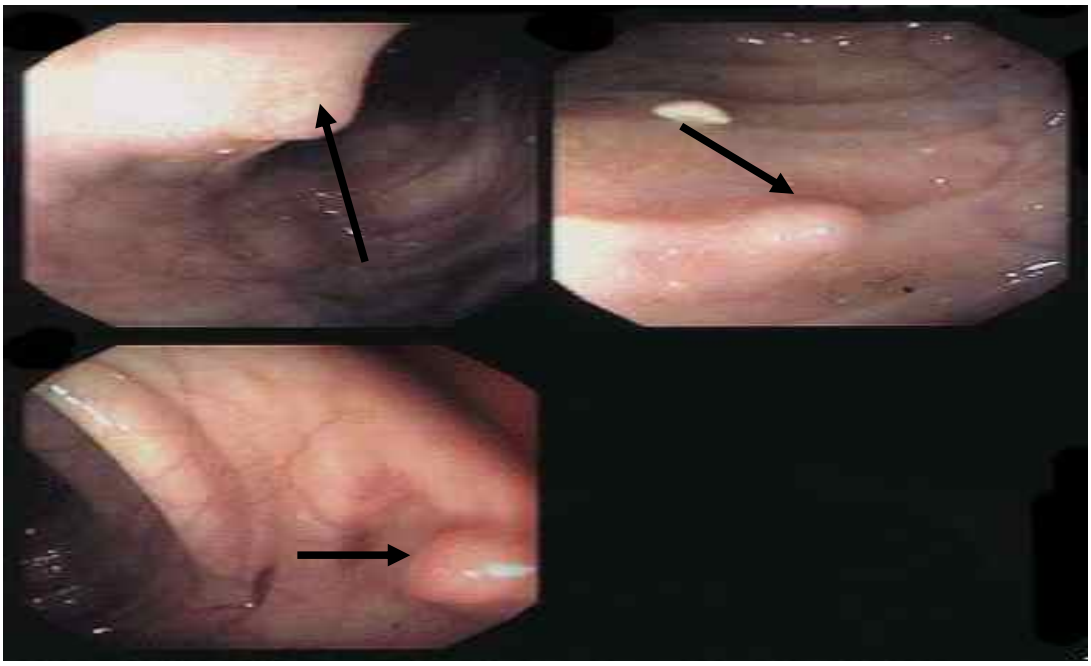


Fig.3: Endoscopic view showing nodules in the duodenum I and II parts.

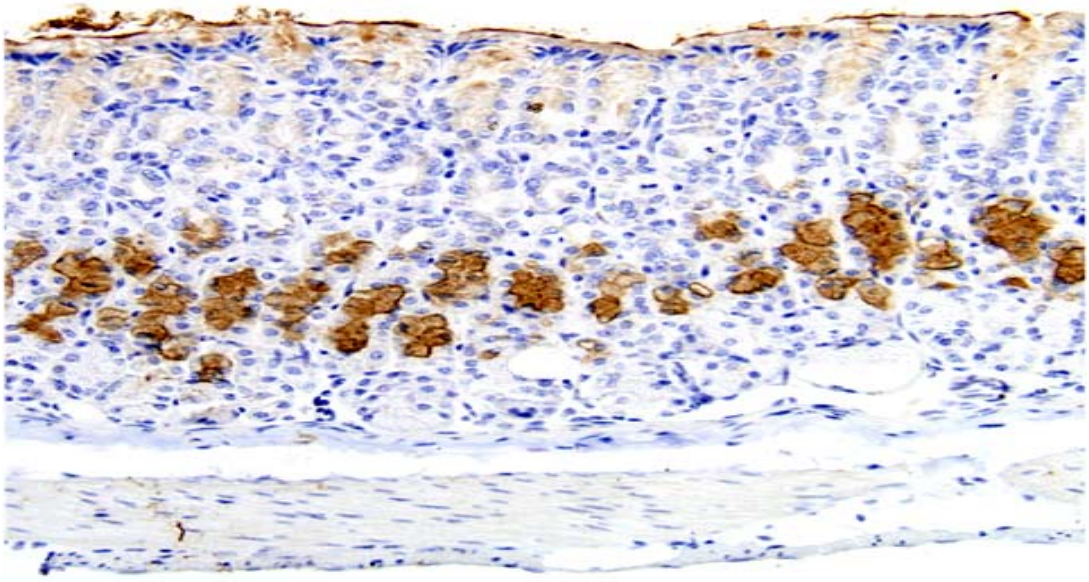


Fig.4: CK AE1/AE3 staining of normal stomach 100X

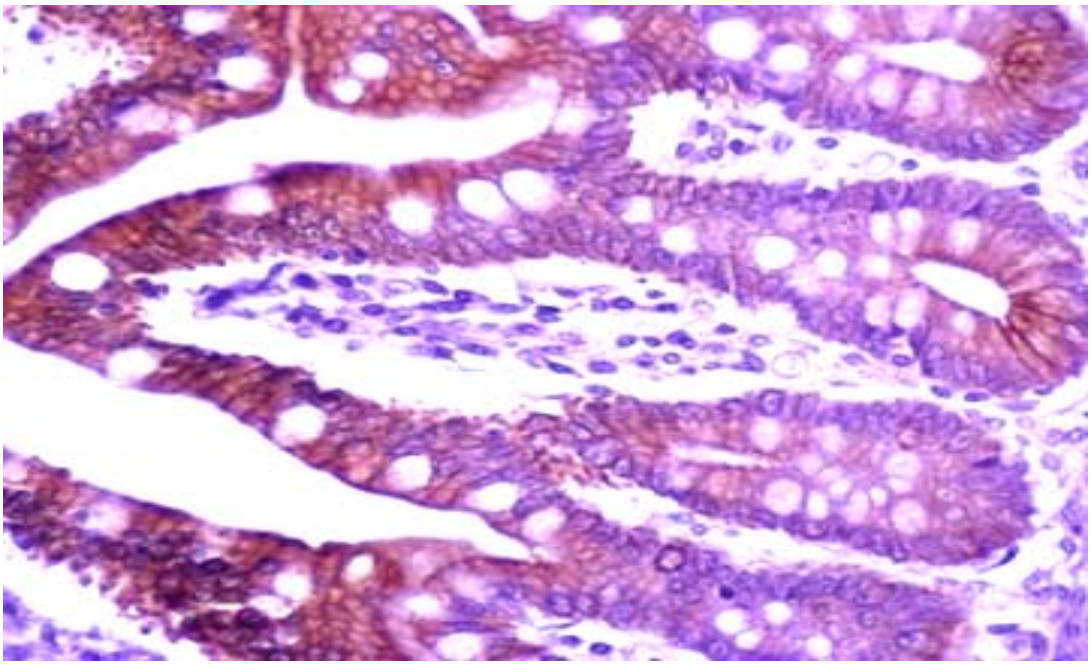


Fig.5: CK AE1/AE3 staining of normal duodenum 400X

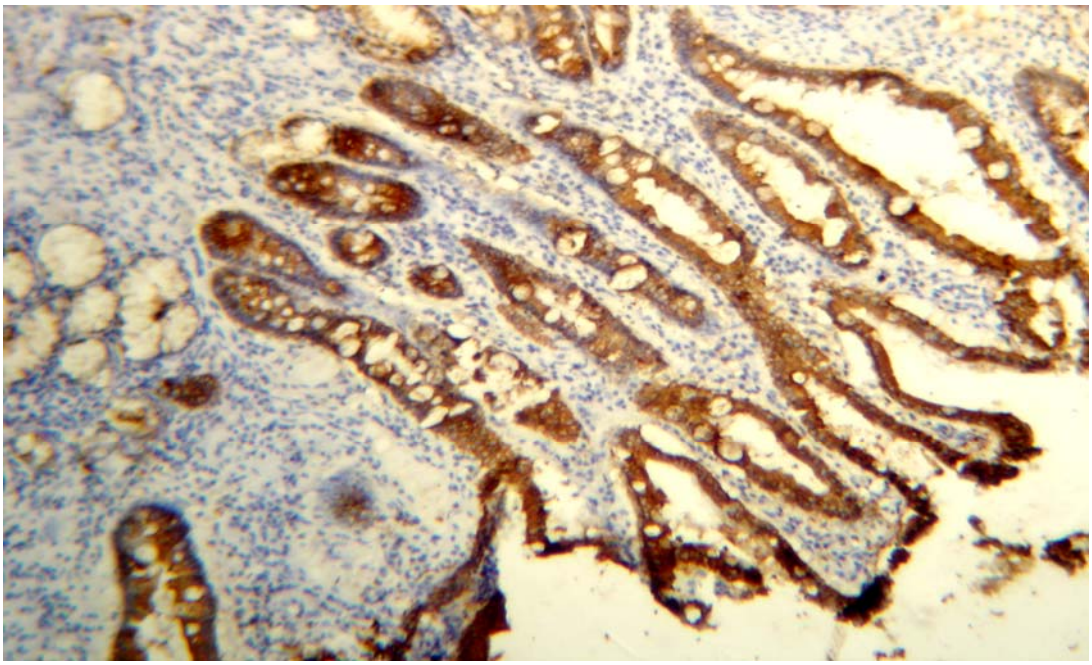


Fig.6: CK AE1/AE3 staining of normal duodenal mucosa 100X

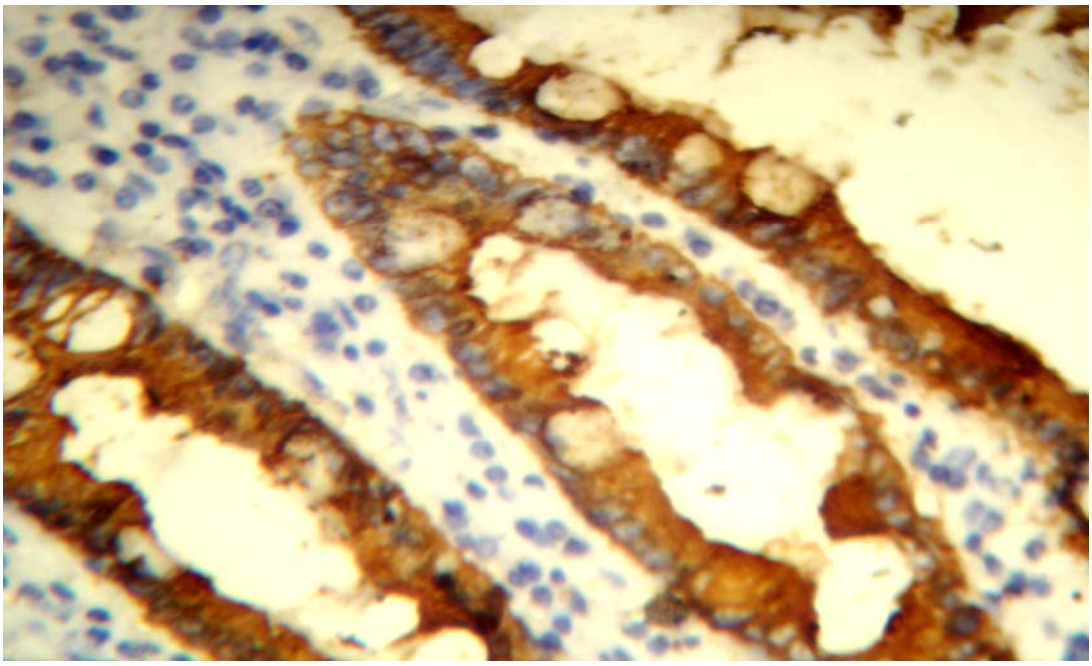


Fig.7:CK AE1/AE3 staining of normal small intestinal glands 400X

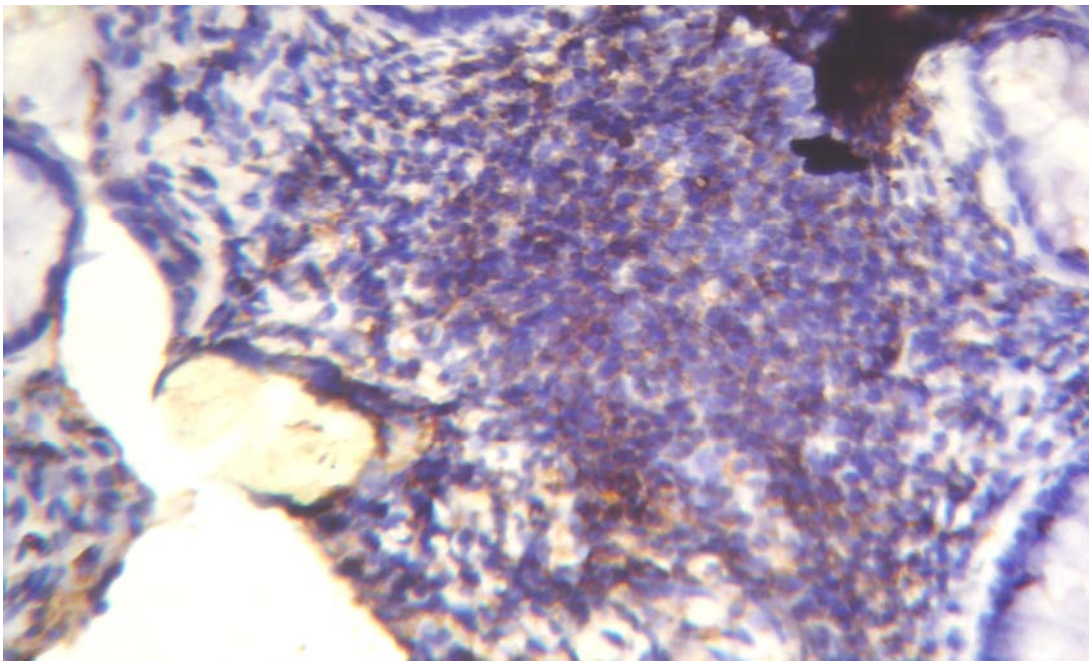


Fig. 8: CD 20 positive cells dispersed mostly in marginal zone in normal Payer's patch 400X

Group 1: Those patients who have had their biopsies confirmed to be lymphoma

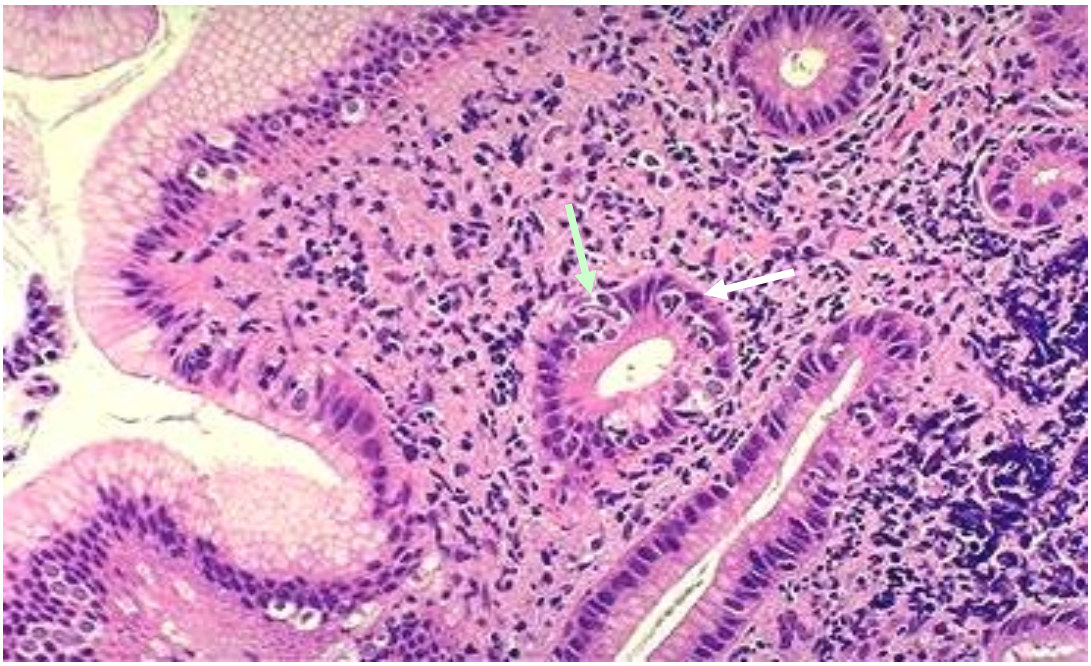


Fig.9: Maltoma stomach showing lymphoepithelial lesion 400X

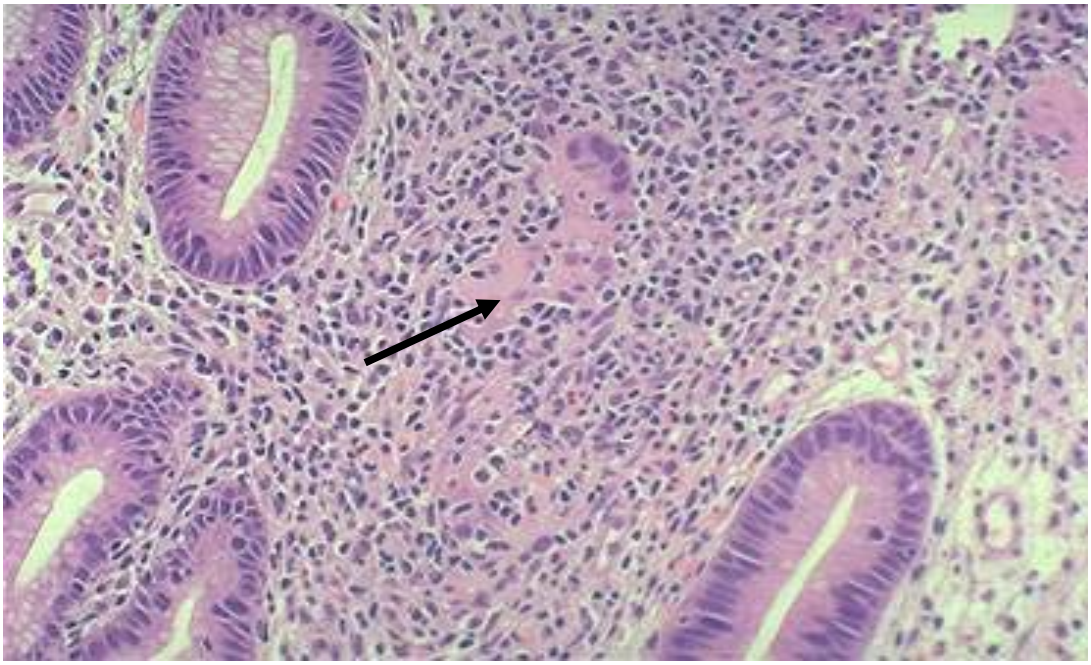


Fig.10: Maltoma stomach showing lymphoepithelial lesion 400X

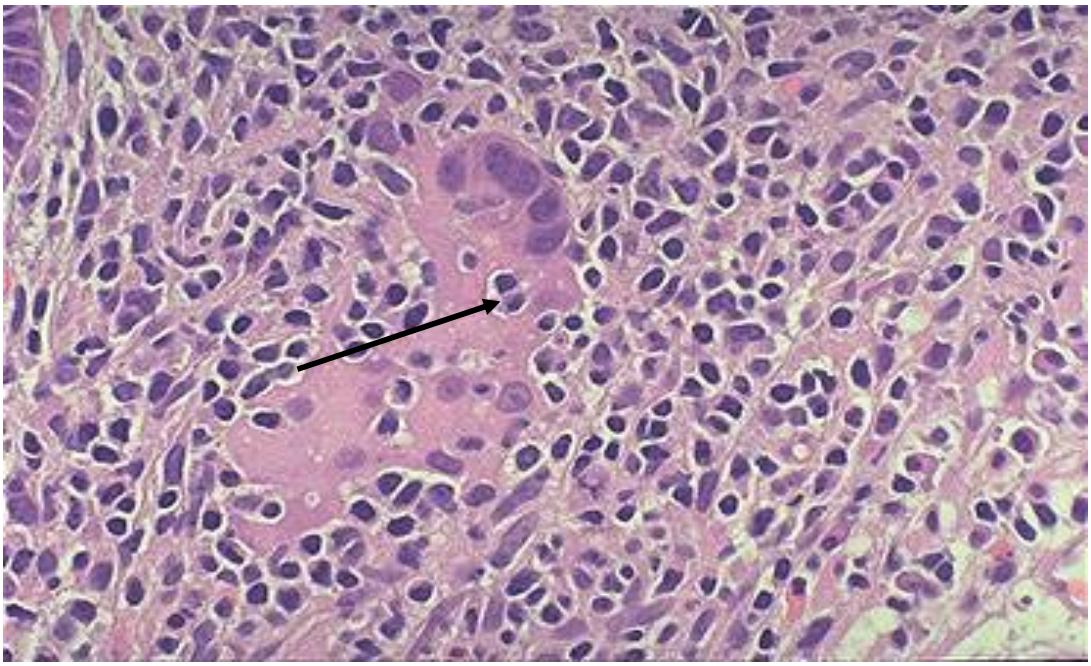


Fig.11: Maltoma stomach showing lymphoepithelial lesion 400X

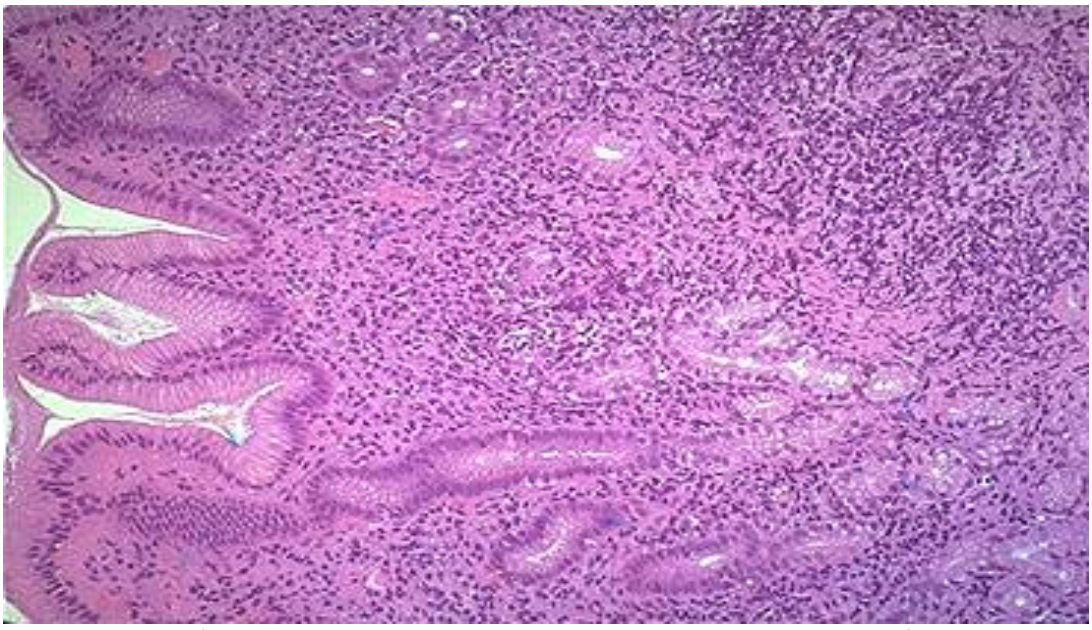


Fig.12: Section from gastric mucosa showing dense lymphoplasmacytic infiltrate in the lamina propria raising the suspicion of lymphoma.100X

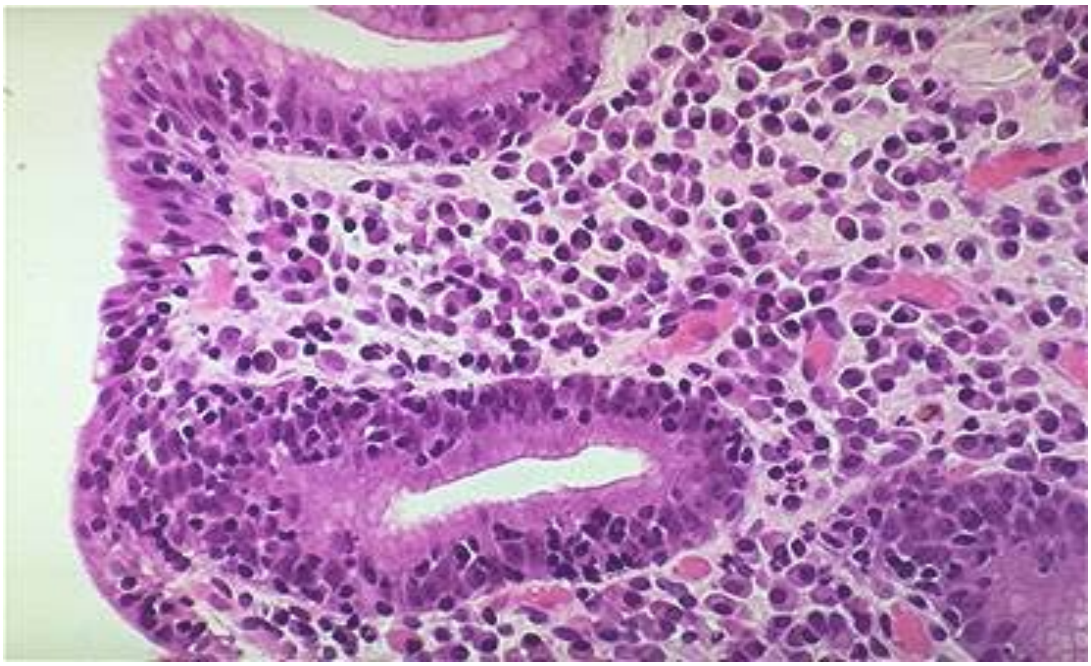


Fig.13: Section from gastric mucosa showing dense lymphoplasmacytic infiltrate in the lamina propria raising the suspicion of lymphoma in the same specimen in Fig.12 400X

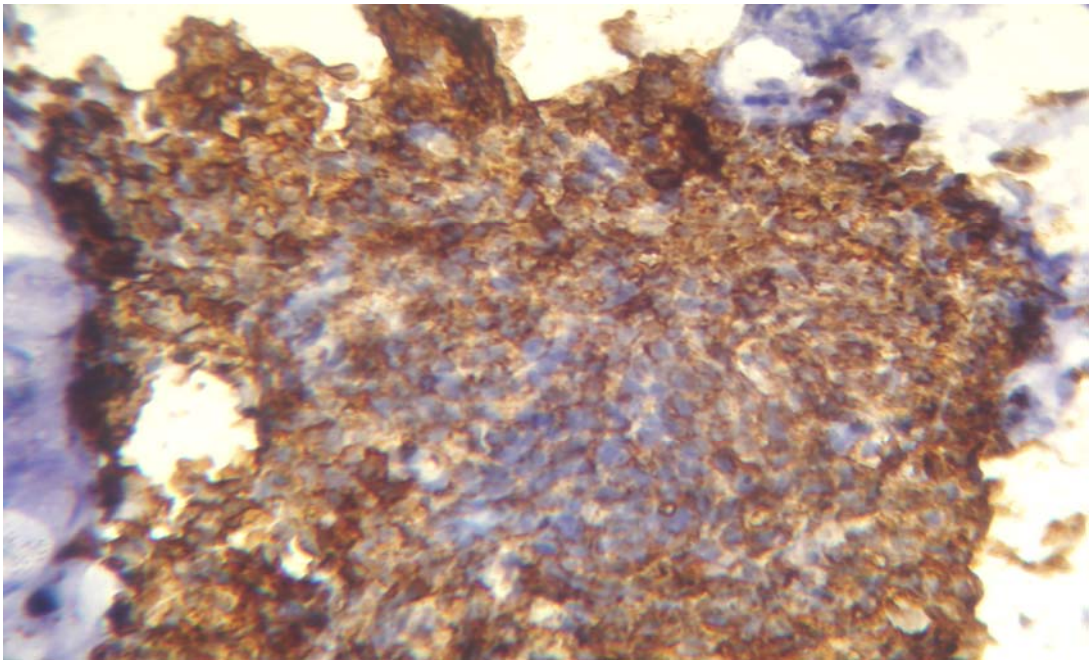


Fig.14: Follicular colonization of CD 20 positive centrocyte like cells in a case of Gastric Lymphoma in the same specimen in Fig.12 400X

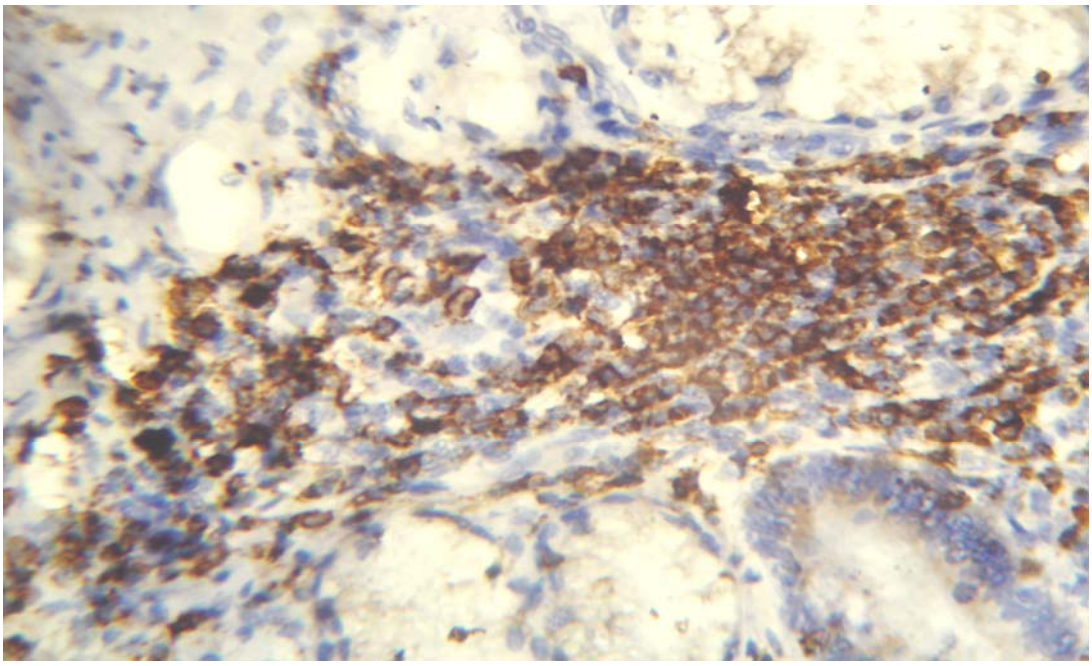


Fig.15: Follicular colonization and melting away of CD 20 positive cells in gastric lymphoma in the same specimen in Fig.12 400X

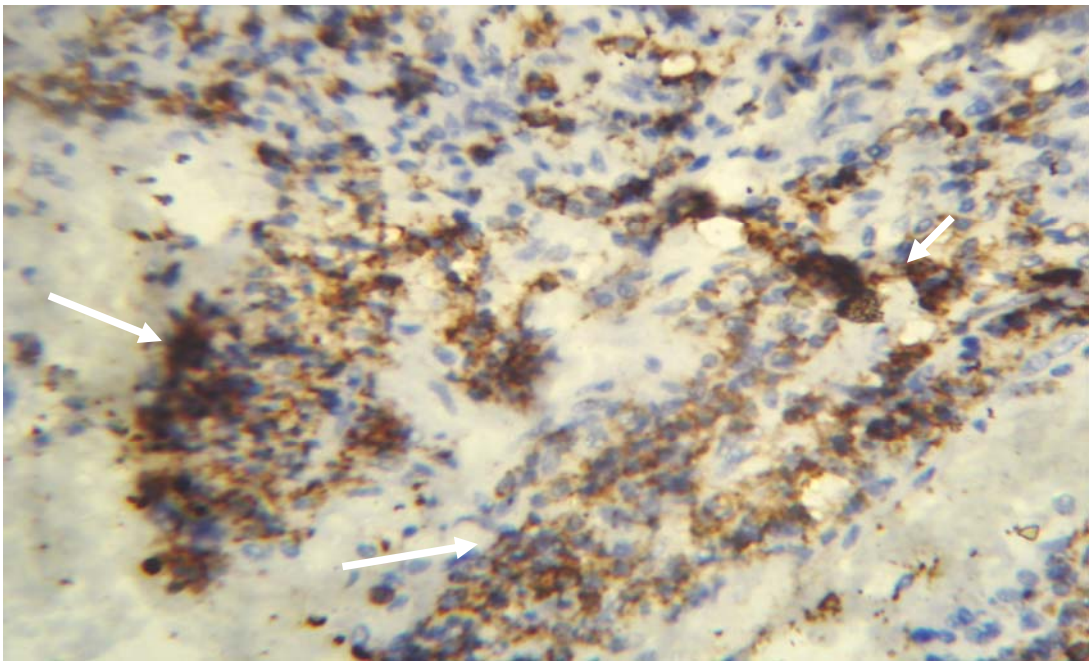


Fig.16: Section shows glands completely destroyed by CD 20 positive lymphoma cells in the same specimen in Fig.12 400X

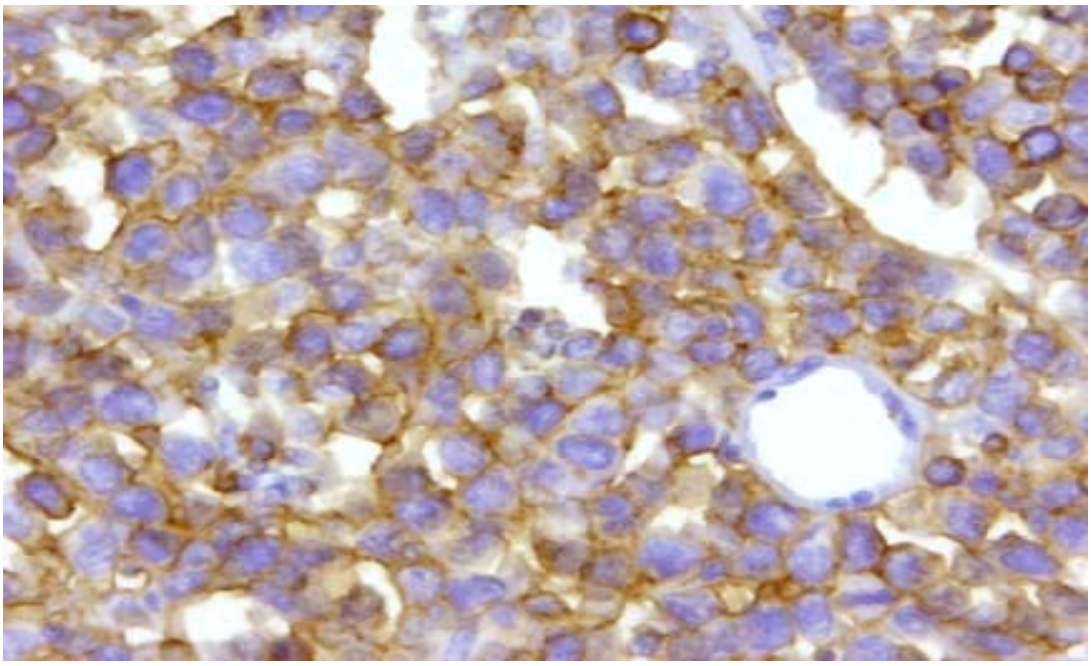


Fig.17: Lymphoma cells in gastric biopsy showing diffuse positive staining with CD 20 in the same specimen in Fig.12 400X

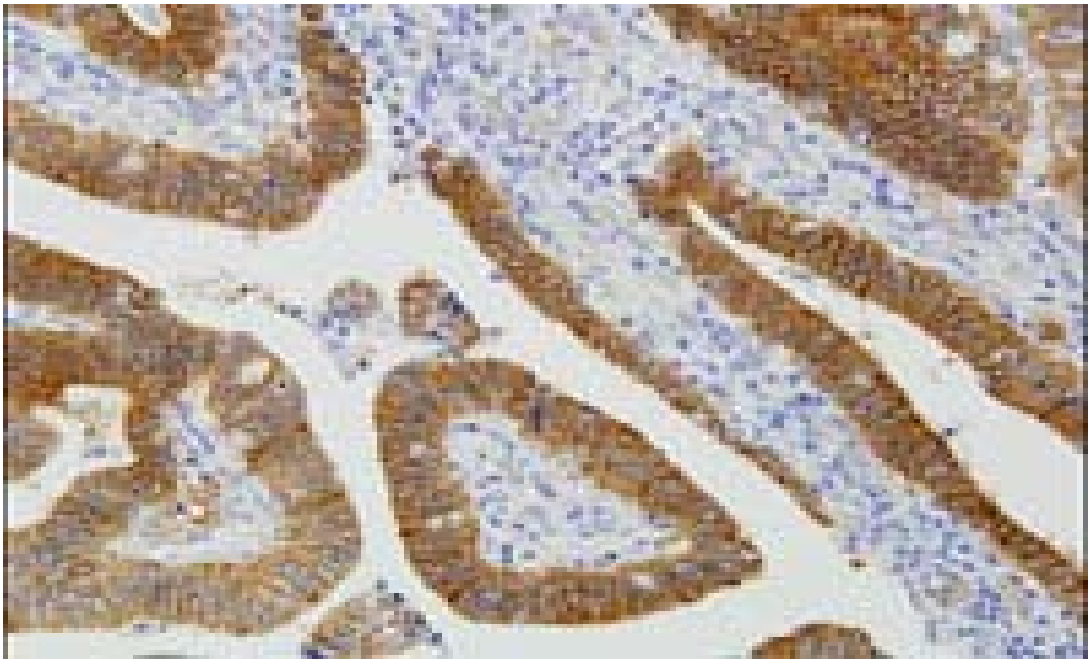


Fig.18: Lymphoma cells showing negative staining with CK AE1/AE3 in the same specimen in Fig.12 400X

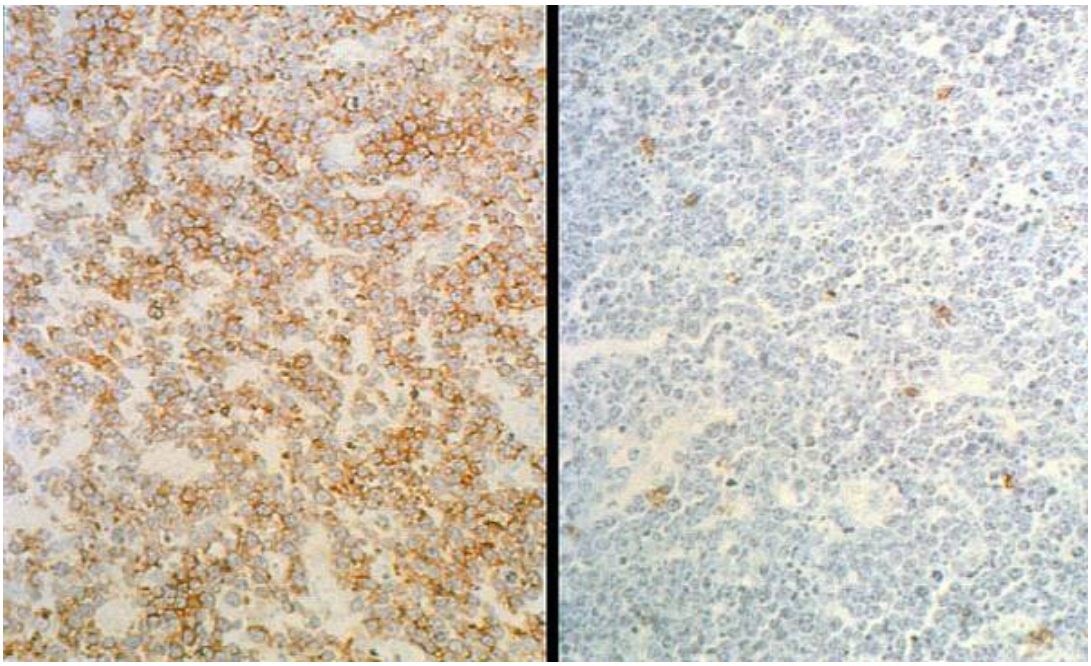


Fig.19: Section on the left shows CD 20 positive cells and on the right shows CD 3 negative cells in a case of Gastric Lymphoma in the same specimen in Fig.12 100X

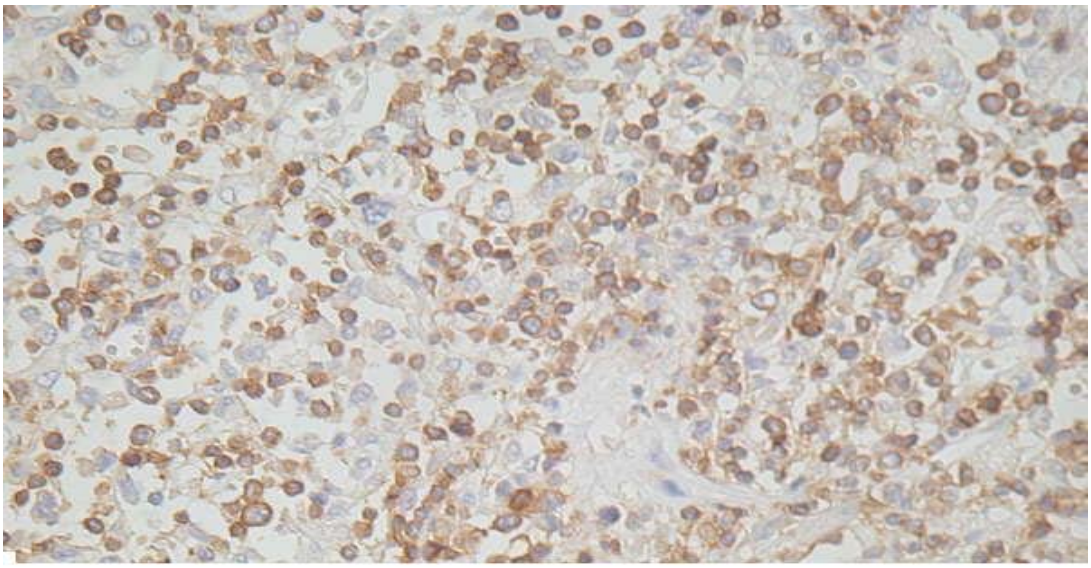


Fig.20: Lymphoma stomach – Cells showing diffuse positivity with CD45 in the same specimen in Fig.12 400X

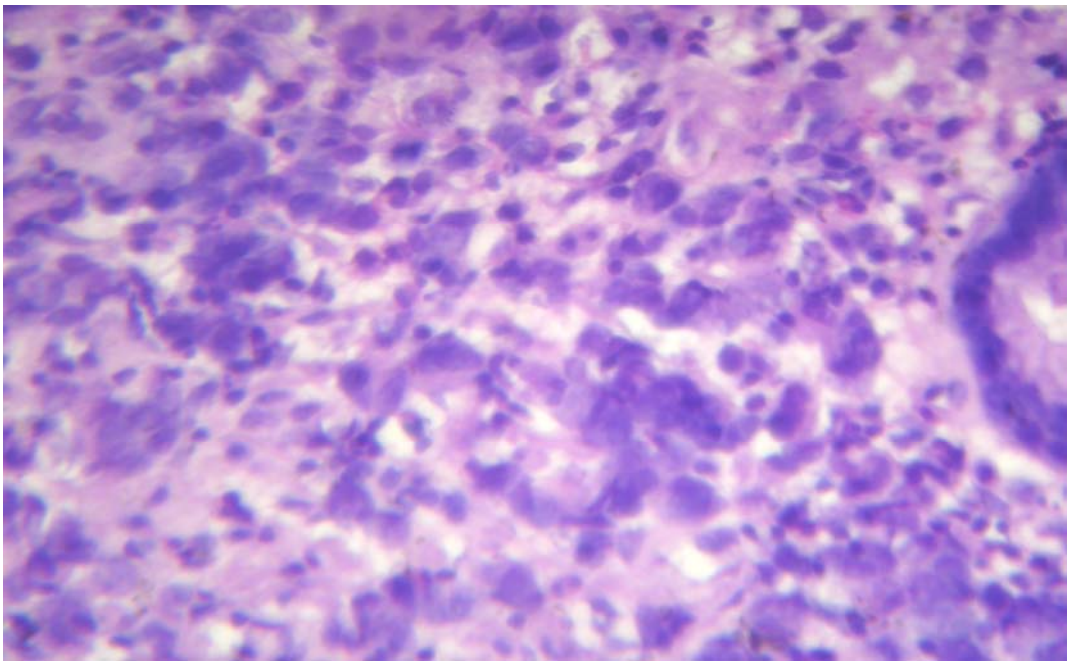


Fig.21: Section shows poorly differentiated carcinoma of the stomach. 400X

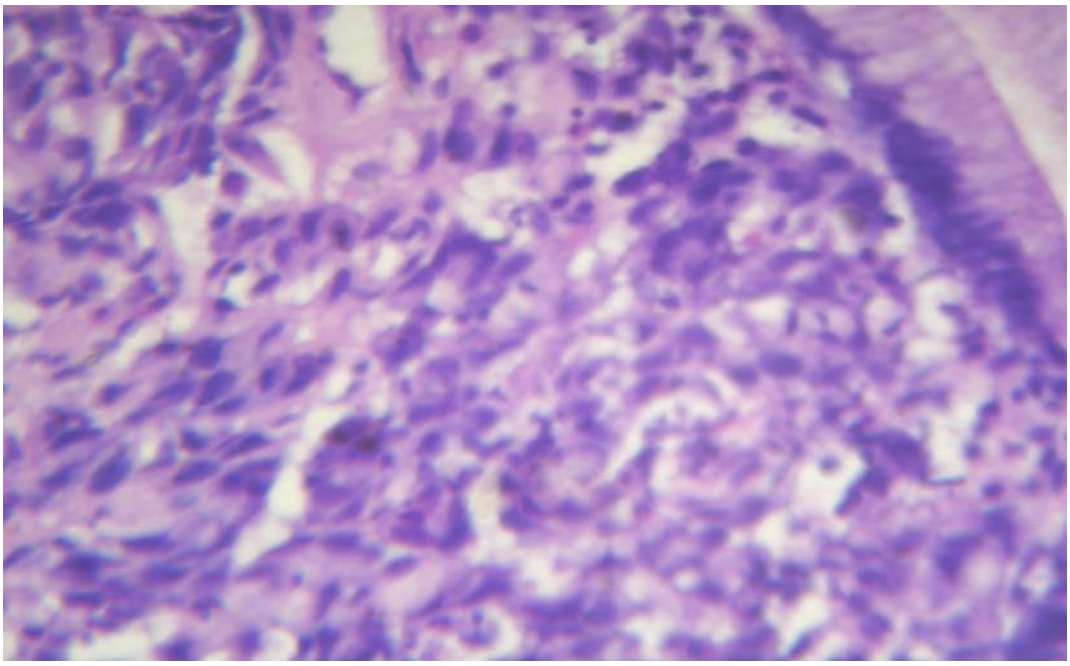


Fig.22: Section shows poorly differentiated carcinoma of stomach.400X

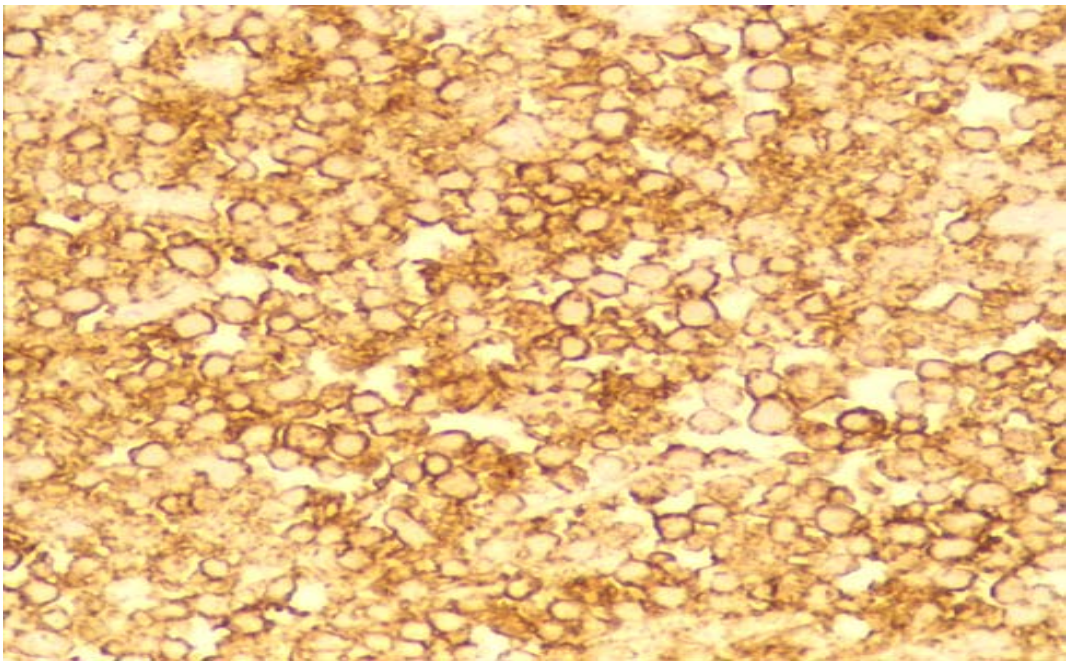


Fig.23: Section shows CK AE1/AE3 positive cells in poorly differentiated carcinoma indicating their epithelial origin (Same specimen as in Fig.22). 400X

Group 2: Those cases which have been confirmed to be benign inflammatory lesions.

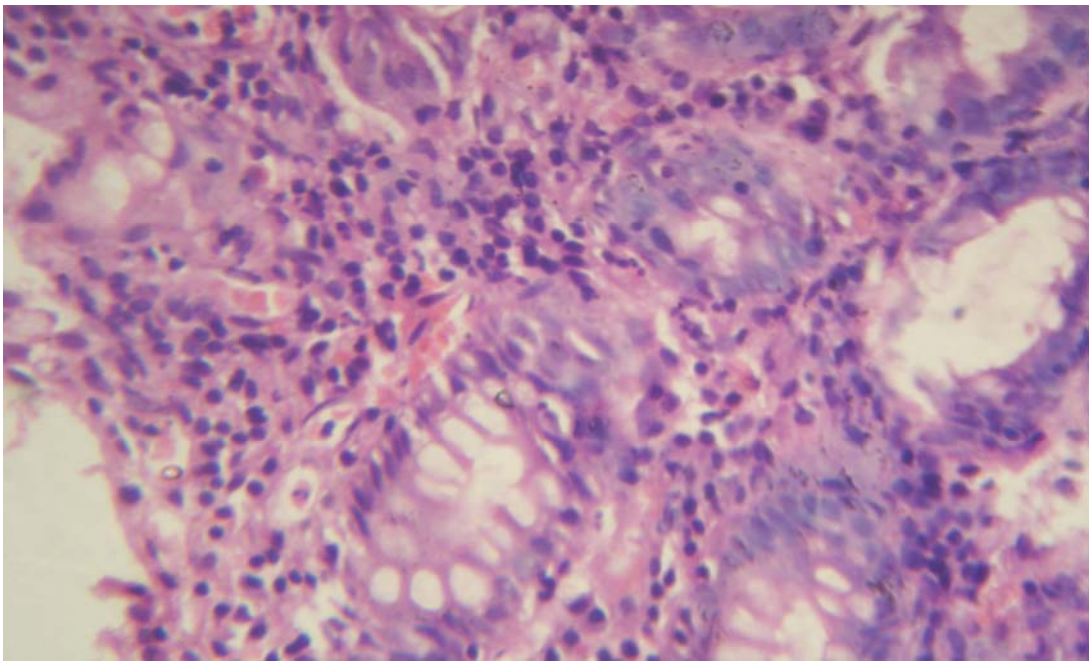


Fig.24: Section from gastric mucosa showing dense mononuclear infiltrates in the lamina propria 400X

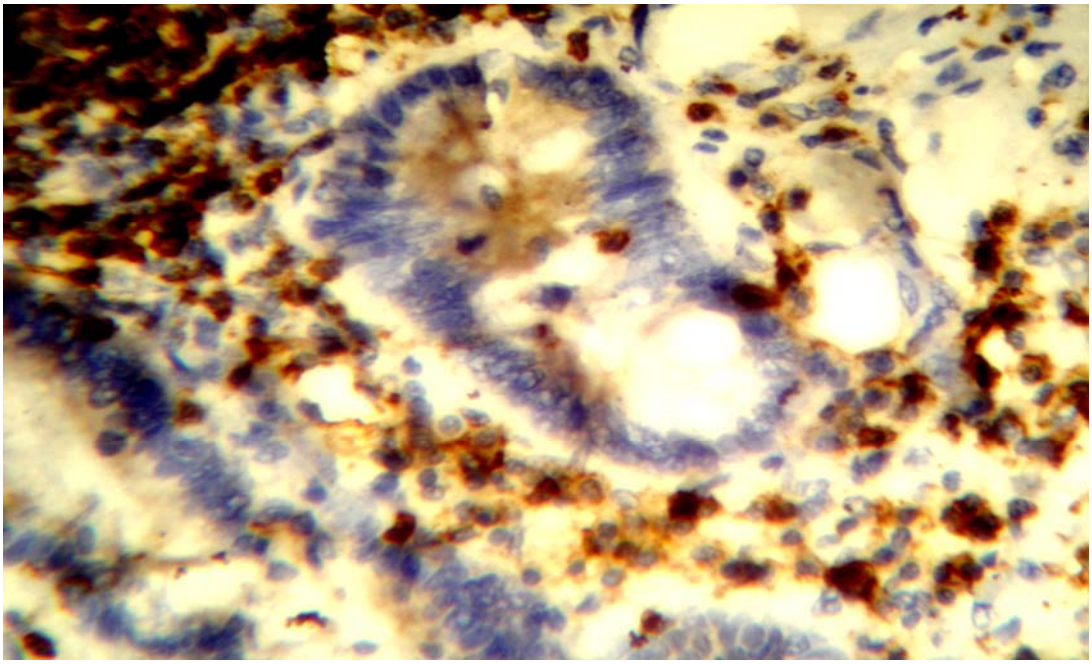


Fig.25: Section shows CD 45 staining of the same case as in Fig.24 with sparse lymphocytes 400X

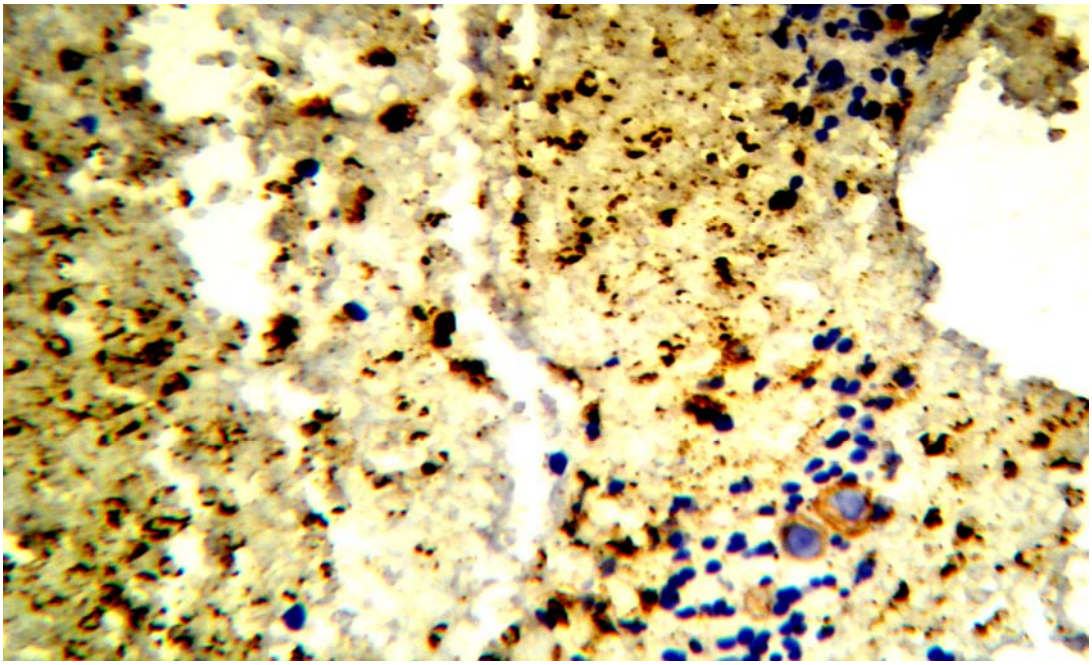


Fig.26: Section shows CD 3 staining of the same case as in Fig.24 with sparse T lymphocytes 400X

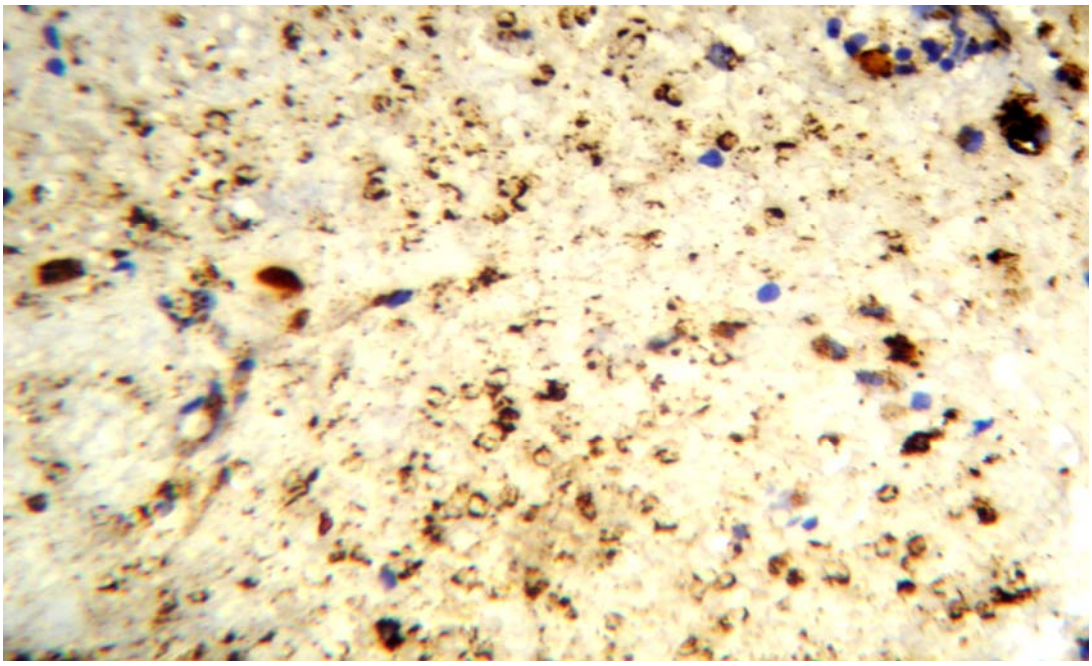


Fig.27: Section shows CD 20 staining of the same case as in Fig.24 with sparse B lymphocytes 400X

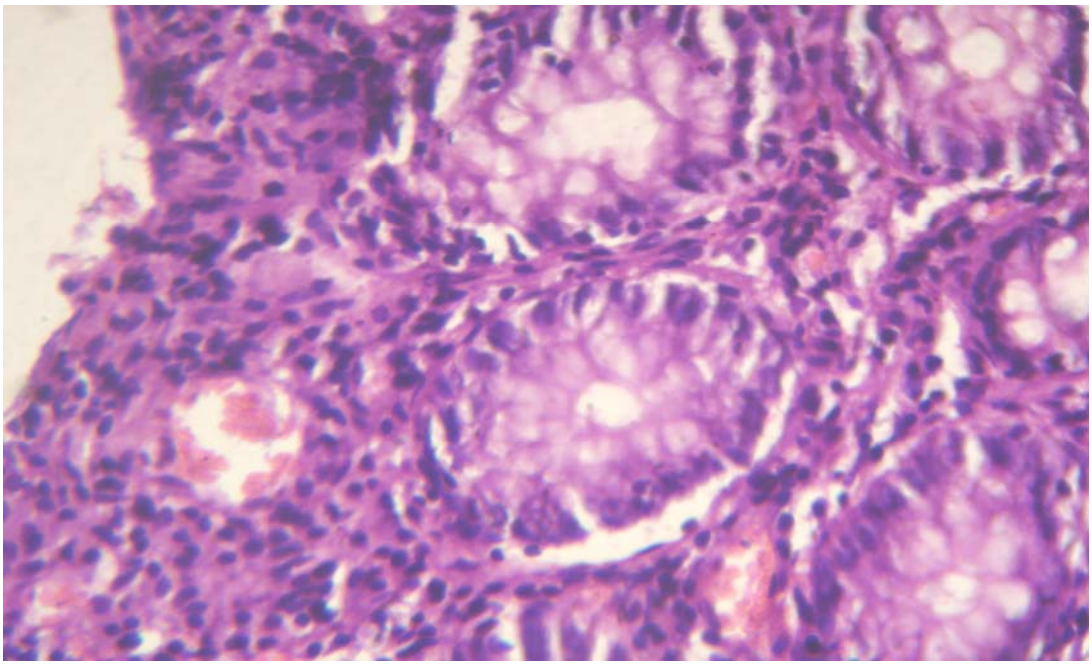


Fig.28: Section shows duodenal mucosa with dense mononuclear infiltrate in the lamina propria 400X

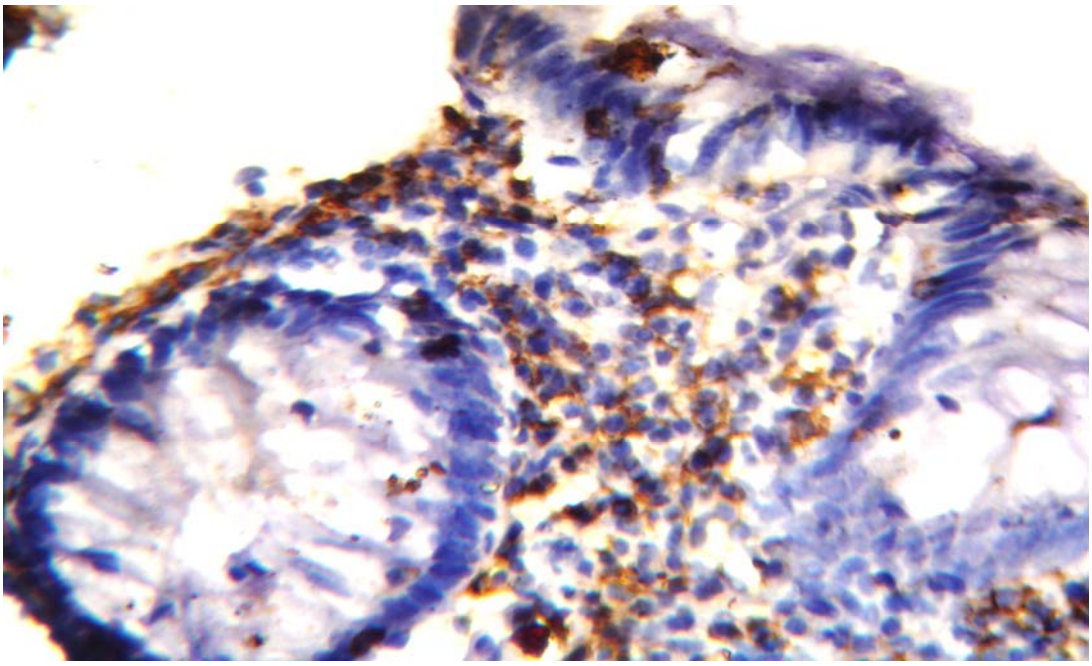


Fig.29: Section from the same duodenal mucosa as in Fig.28 shows sparse CD 20 positive cells without increase in the intraepithelial lymphocytes.400X

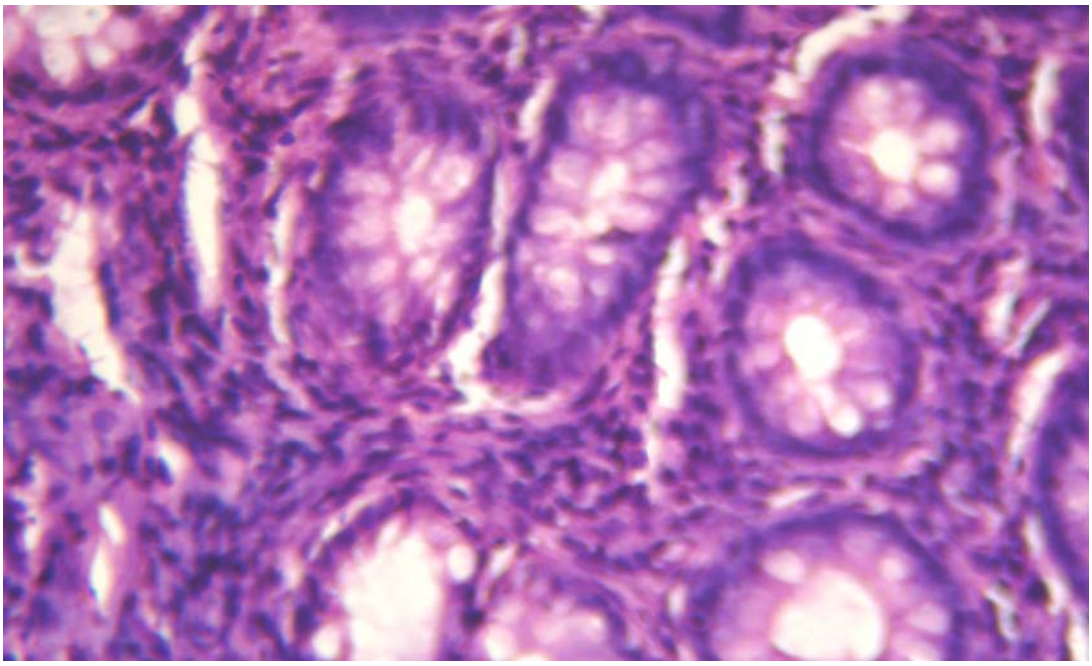


Fig.30: Section from the colonic mucosa showing dense lymphoplasmacytic infiltrate in the lamina propria 400X

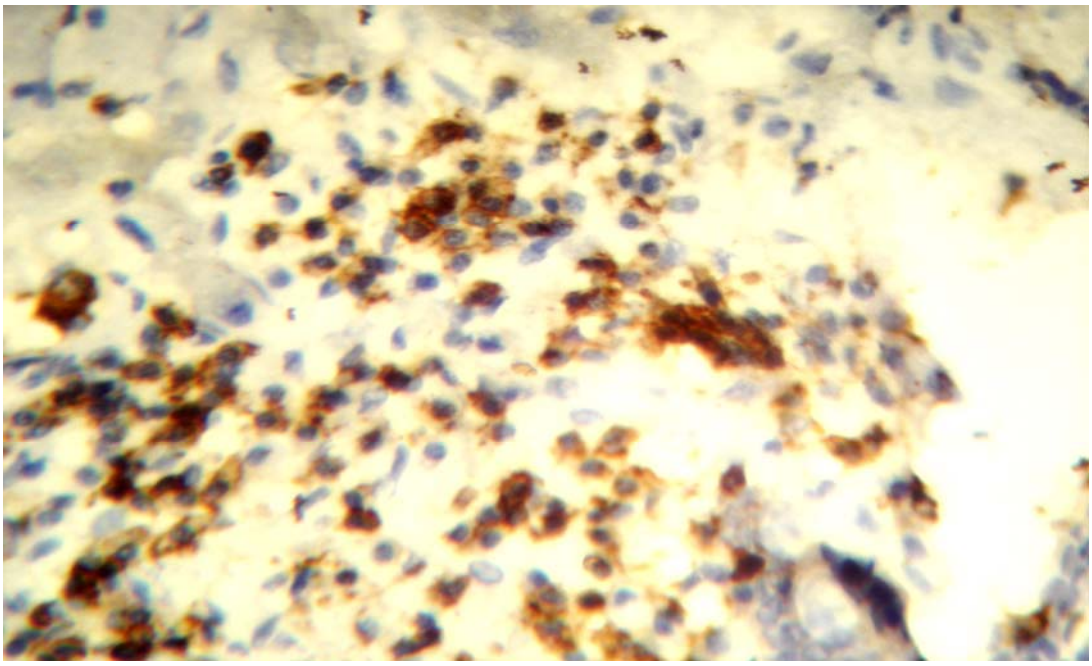


Fig.31: Section shows the same colonic mucosa as in Fig.30 showing CD 20 positive cells without increase in the intraepithelial lymphocytes 400X

Group 3: This group comprises the cases which could not be labeled as lymphoma or neglected as inflammatory pathology based on routine H & E stained sections.

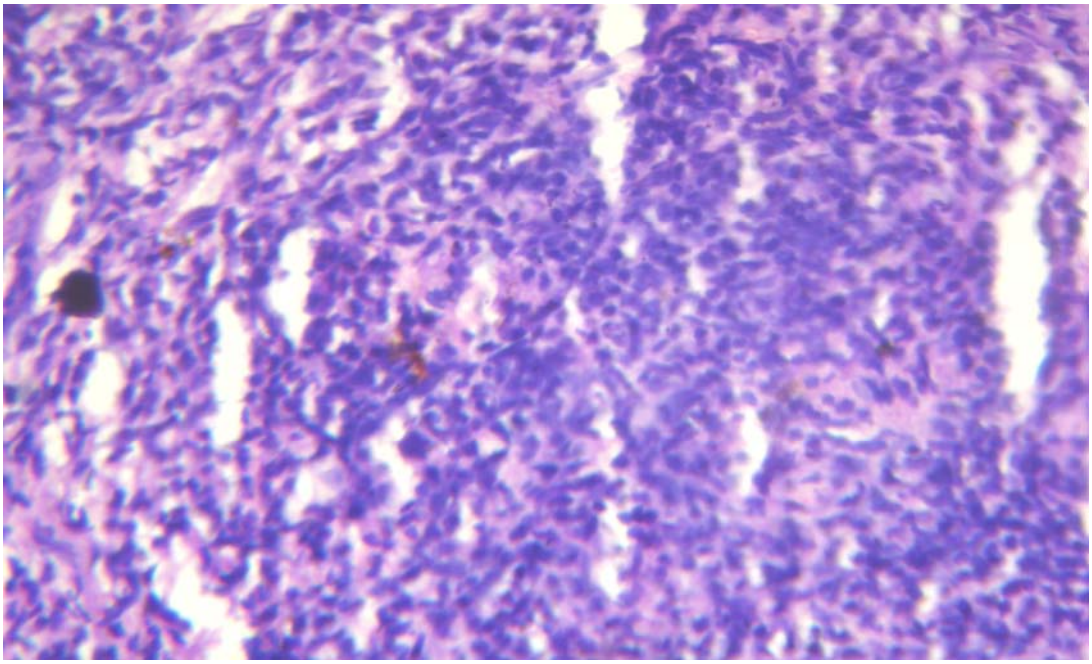


Fig.32: Gastric mucosal biopsy showing diffuse lymphoplasmacytic infiltrates in the lamina propria raising the suspicion of lymphoma 100X

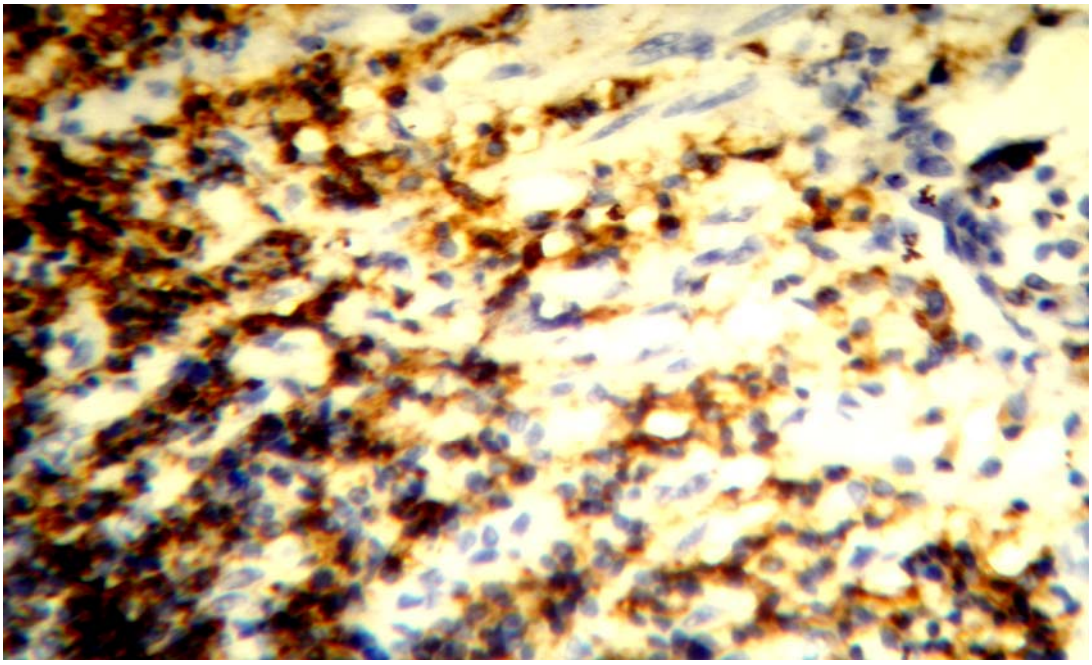


Fig.33: The lymphoplasmacytic infiltrate of the same case as in Fig. 32 staining heavily with CD 20 100X

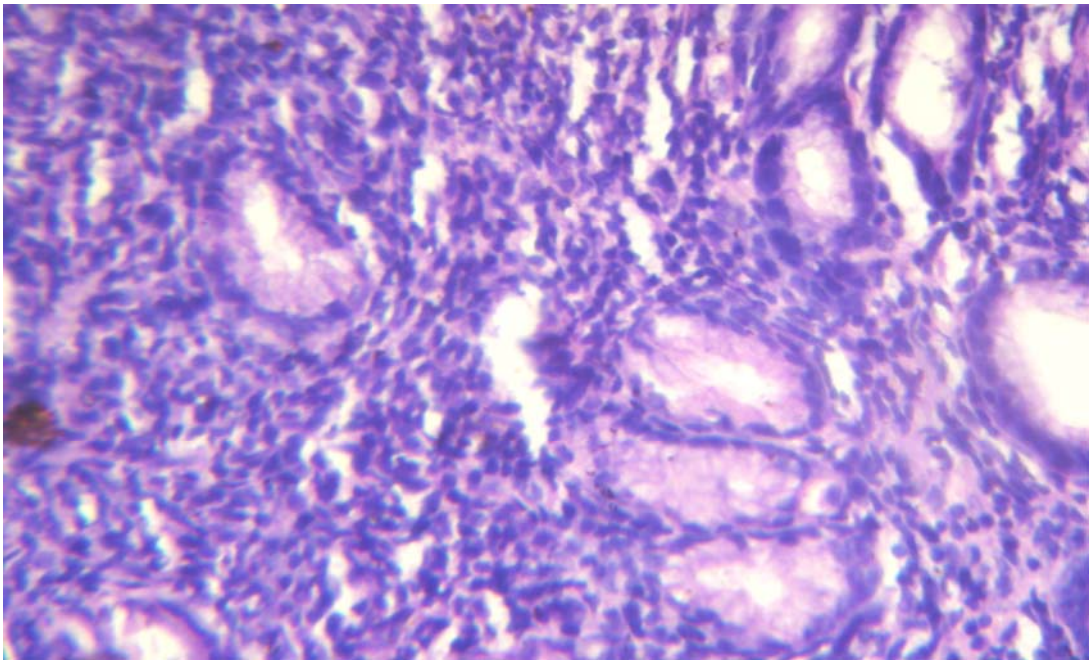


Fig.34: Section from the duodenal mucosa showing diffuse lymphoplasmacytic infiltrate in the lamina propria surrounding the mucosal glands raising the suspicion of lymphoma. 100X

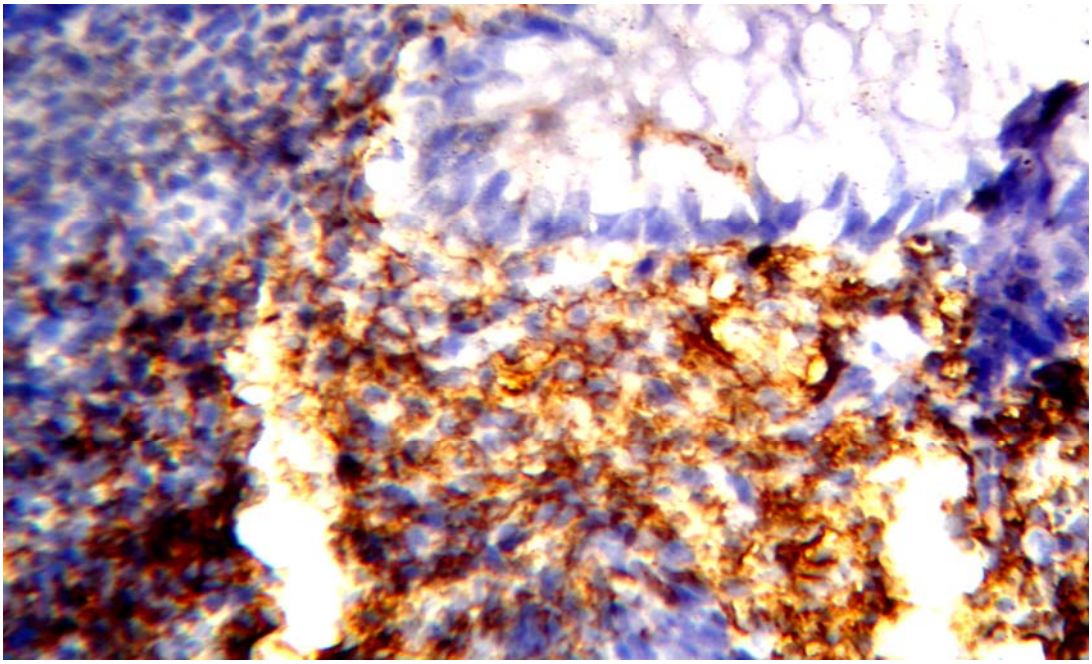


Fig.35: The lymphoplasmacytic infiltrate of the same (Fig.34) staining heavily with CD 20 400X

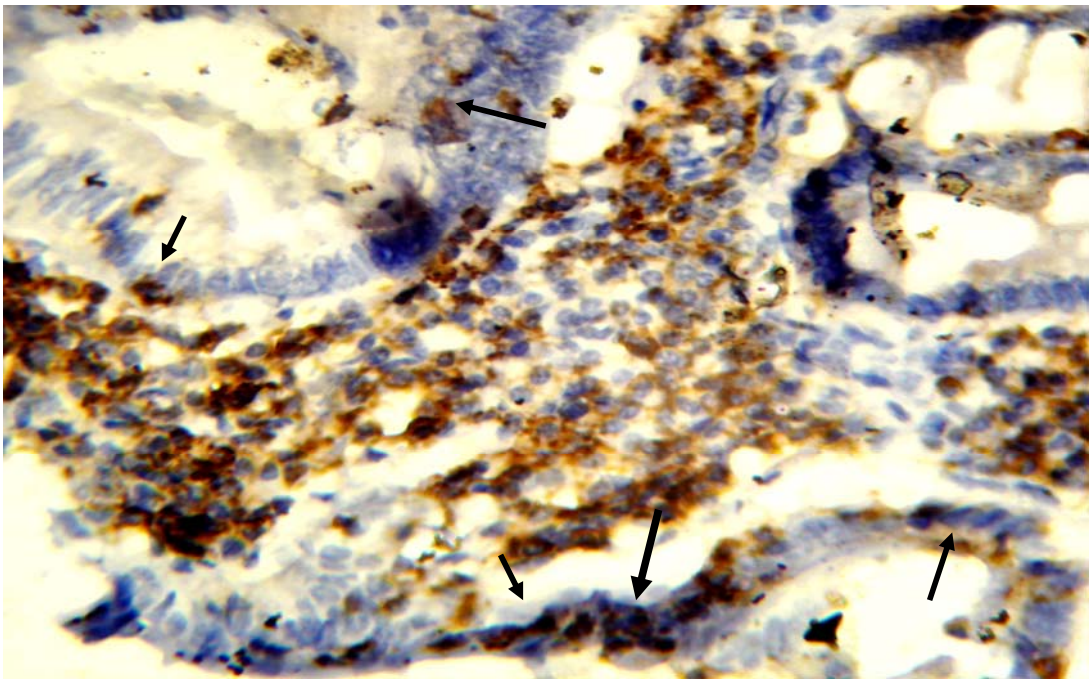


Fig 36: The lymphoplasmacytic infiltrate of the same (Fig.34) staining heavily with CD 20 with increased intra epithelial lymphocytes (more than 20 lymphocytes/100 epithelial cells). 400X

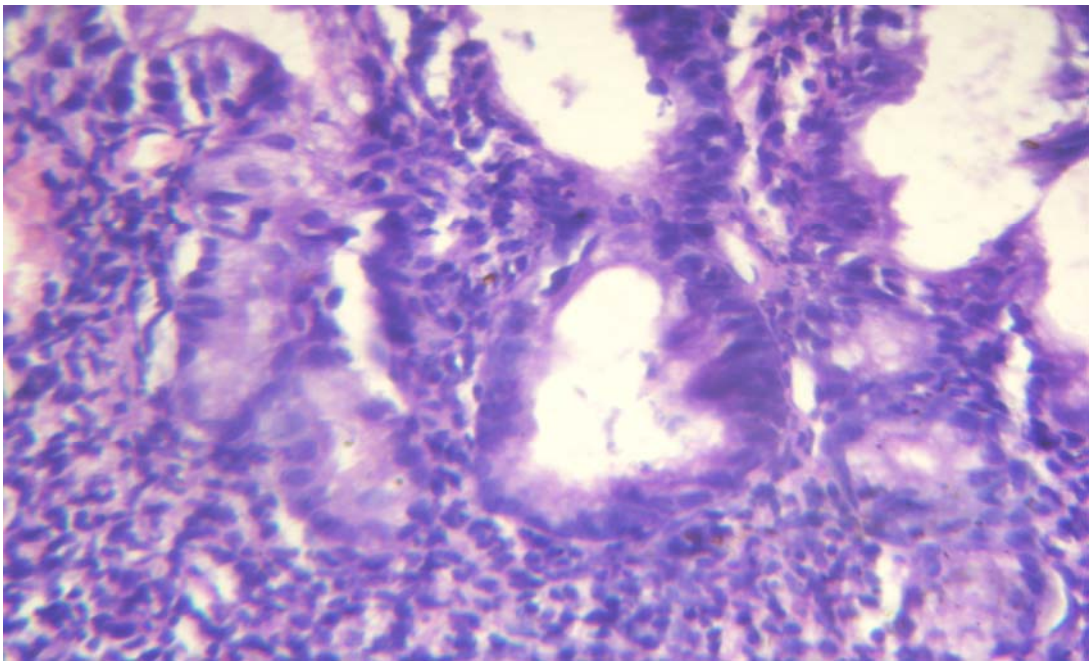


Fig.37: Section from jejunal mucosa showing diffuse lymphoplasmacytic infiltrate in the lamina propria surrounding the mucosal glands raising the suspicion of lymphoma 400x

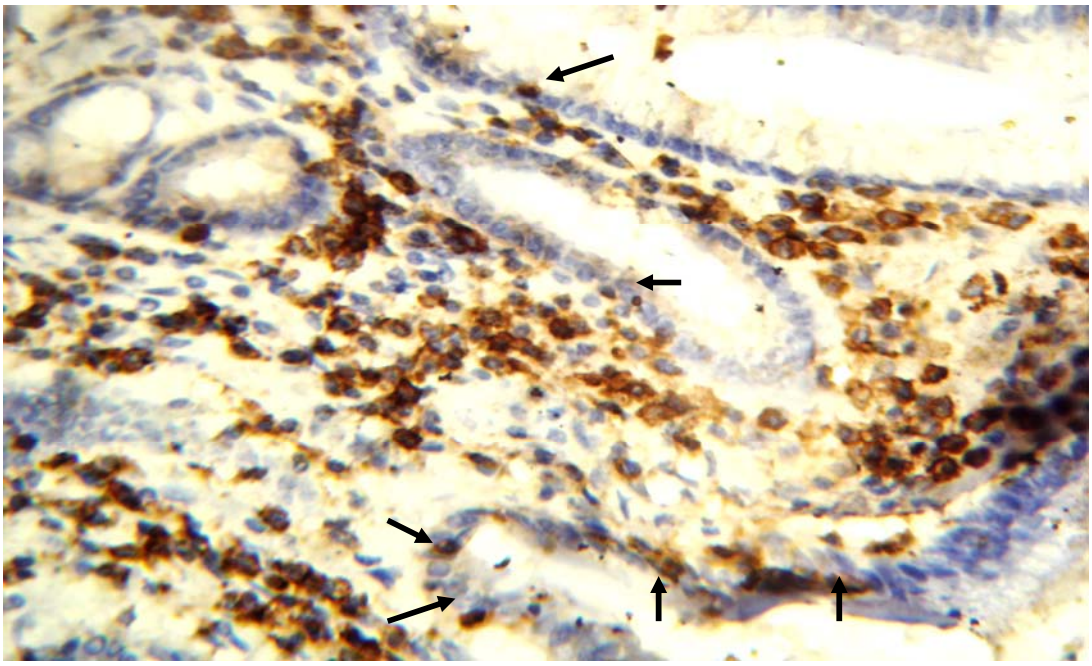


Fig.38: The lymphoplasmacytic infiltrate of the same (Fig.37) staining heavily with CD 20 with increased intraepithelial lymphocytes (arrows).400X

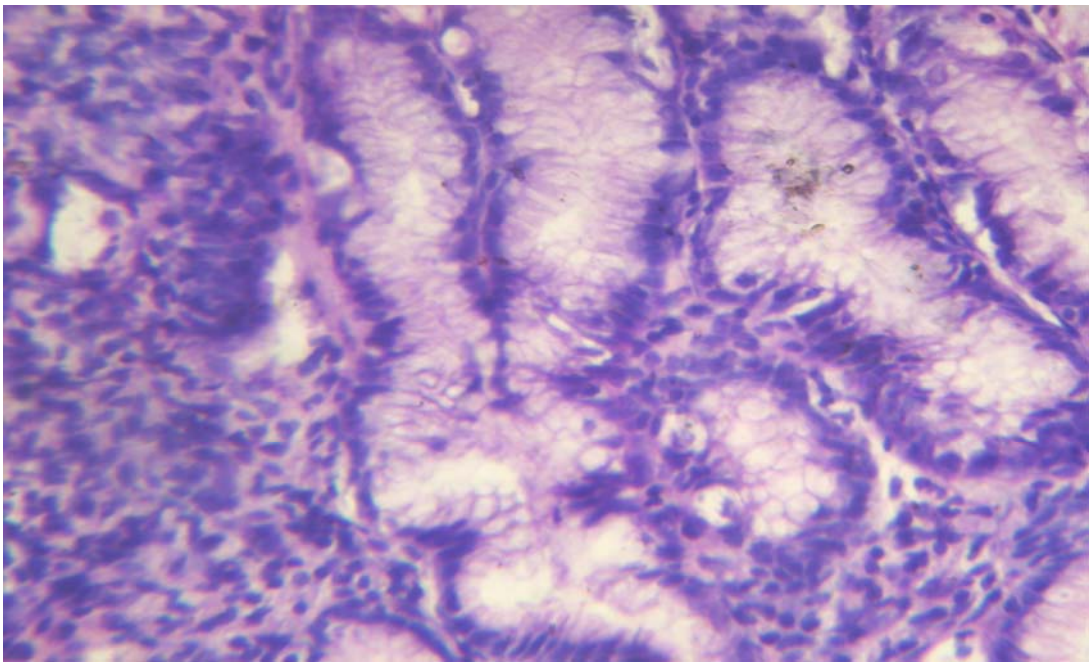


Fig.39: Section from the colonic mucosa diffuse lymphoplasmacytic infiltrate in the lamina propria surrounding the mucosal glands raising the suspicion of lymphoma 400X

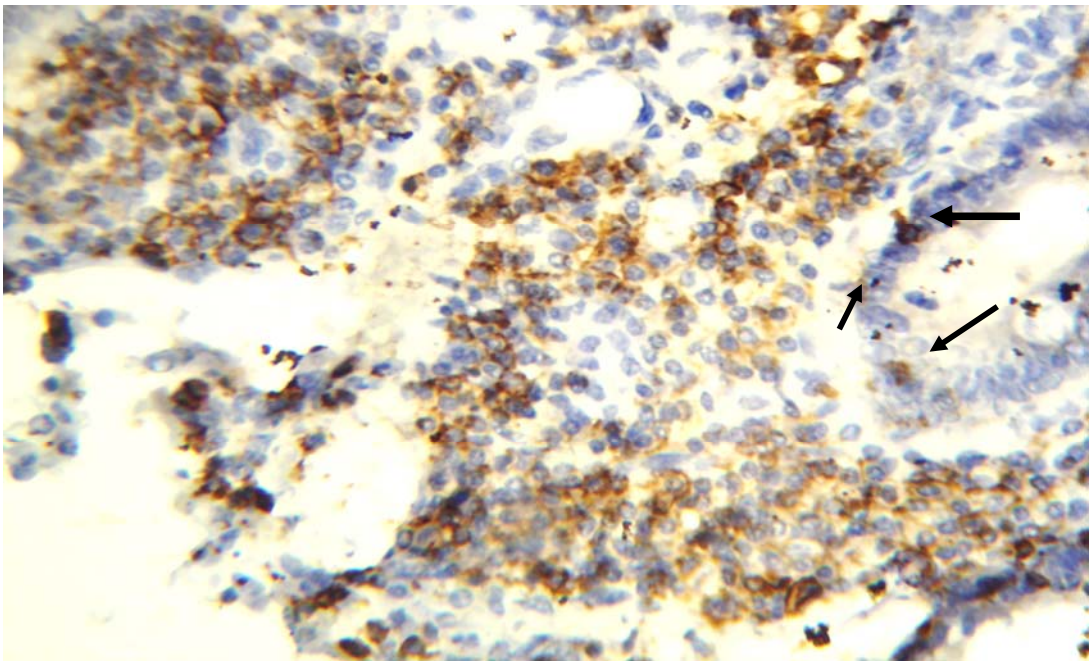


Fig.40: The lymphoplasmacytic infiltrate of the same (Fig.39) staining heavily with CD 20 with increased intraepithelial lymphocytes.400X

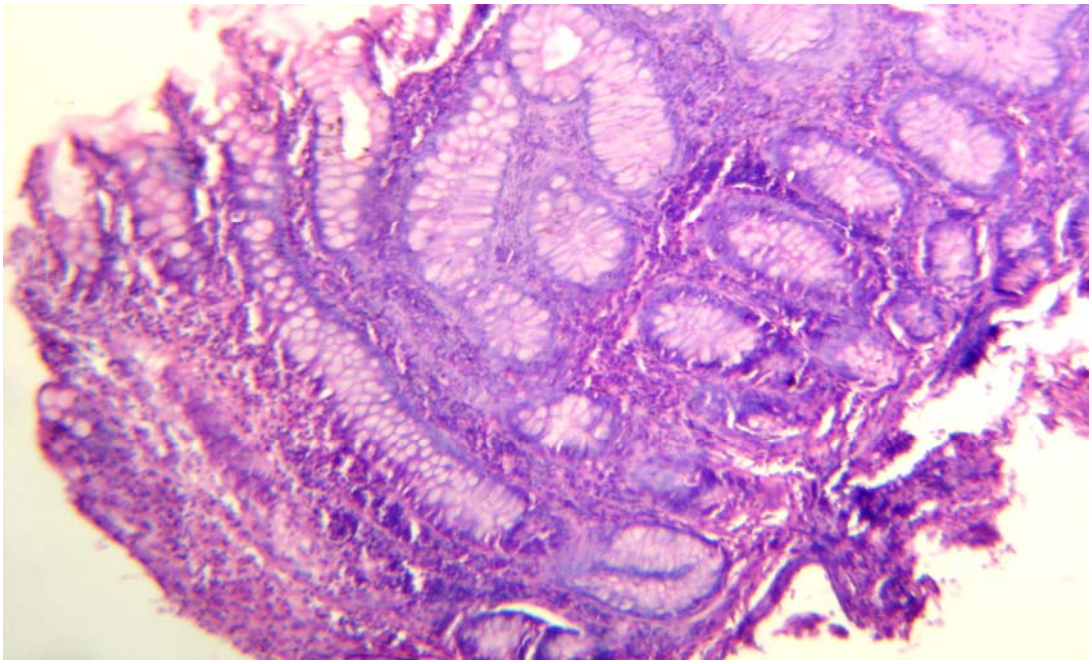


Fig.41: Section from jejunal mucosa showing features of Crohn's disease100X

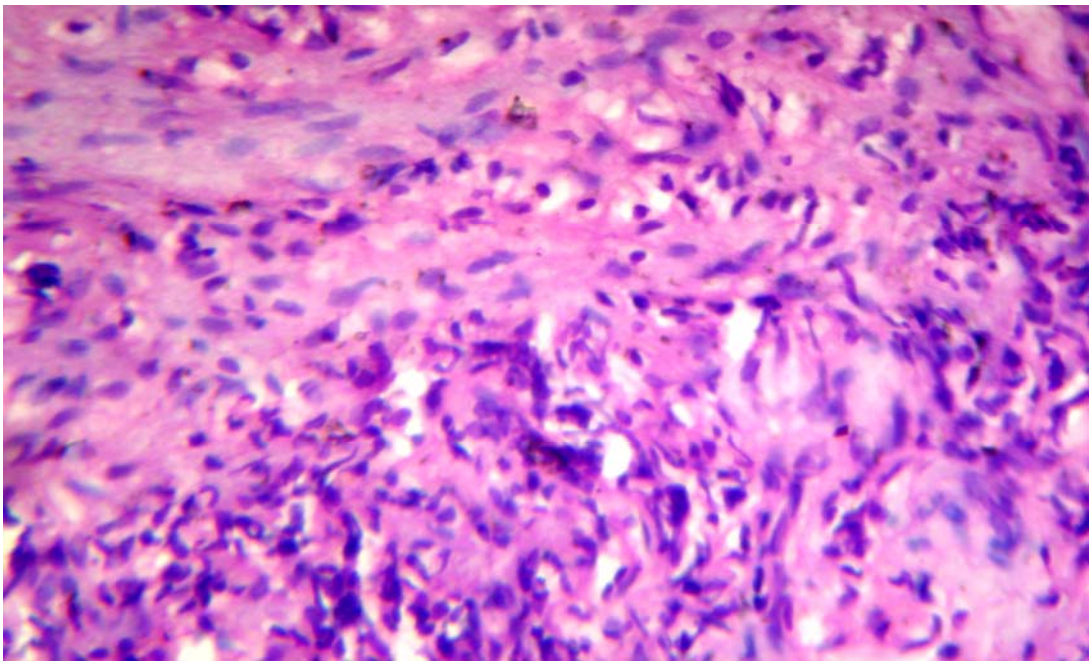


Fig.42: Section from same jejunal mucosa as in Fig.41showing non caseating granuloma of Crohn's disease 400X

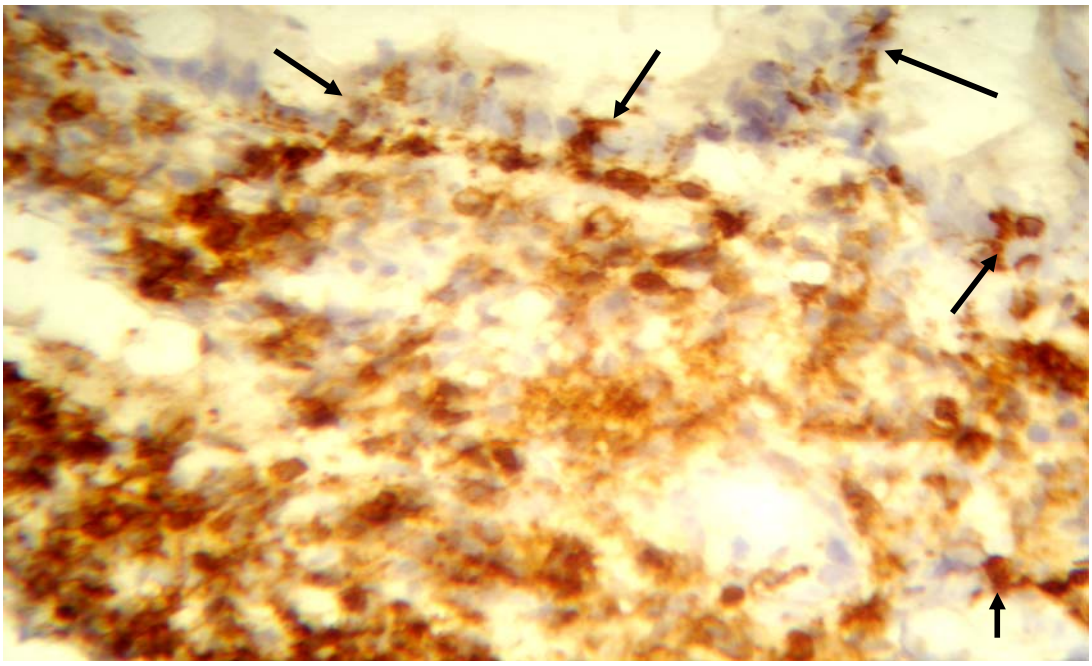


Fig.43: CD 20 positive cells densely populating the lamina propria and increased intraepithelial lymphocytes of the same caseas in Fig.41 400X

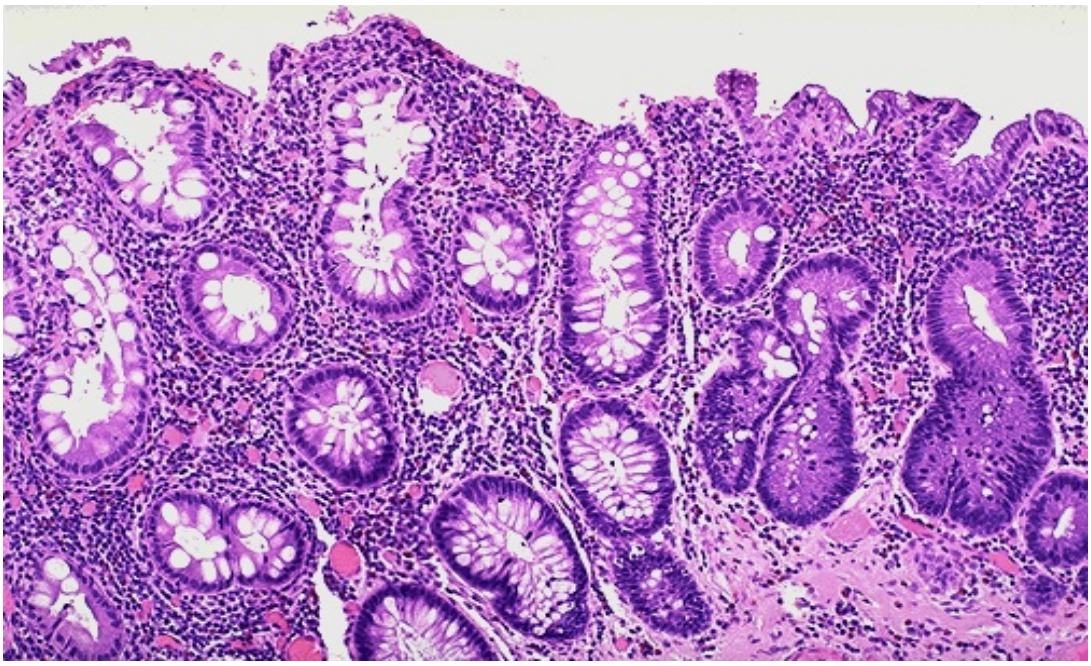


Fig.44: Section shows colonic mucosa with features of Ulcerative Colitis.100X

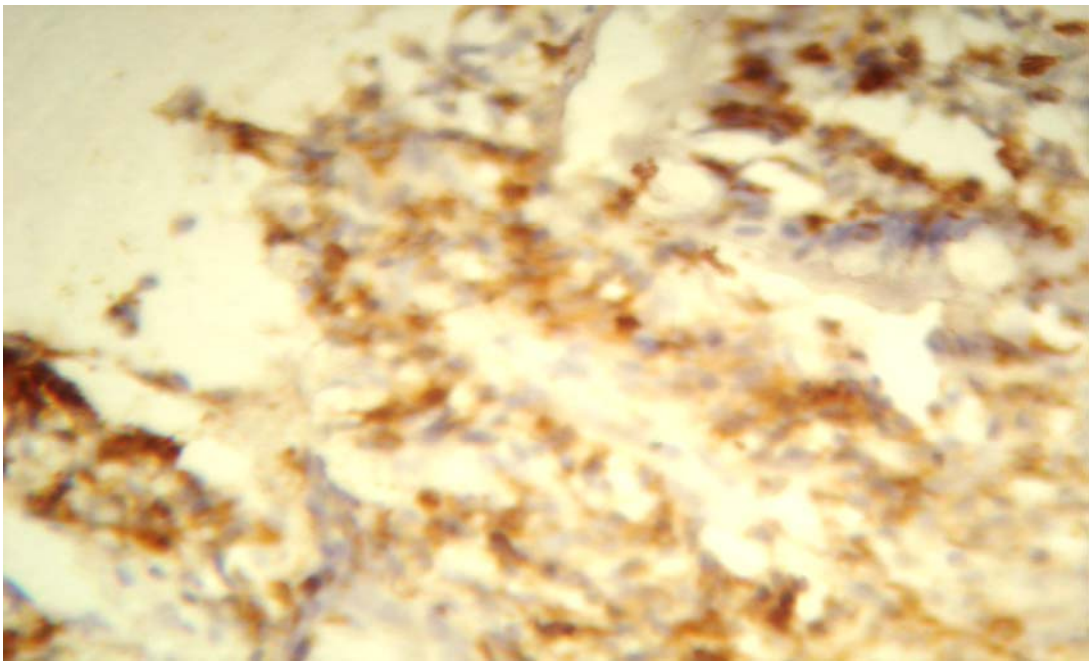


Fig.45: Section shows the same colonic mucosa as in Fig.44 showing CD 45 positive cells without increase in the intraepithelial lymphocytes 400X

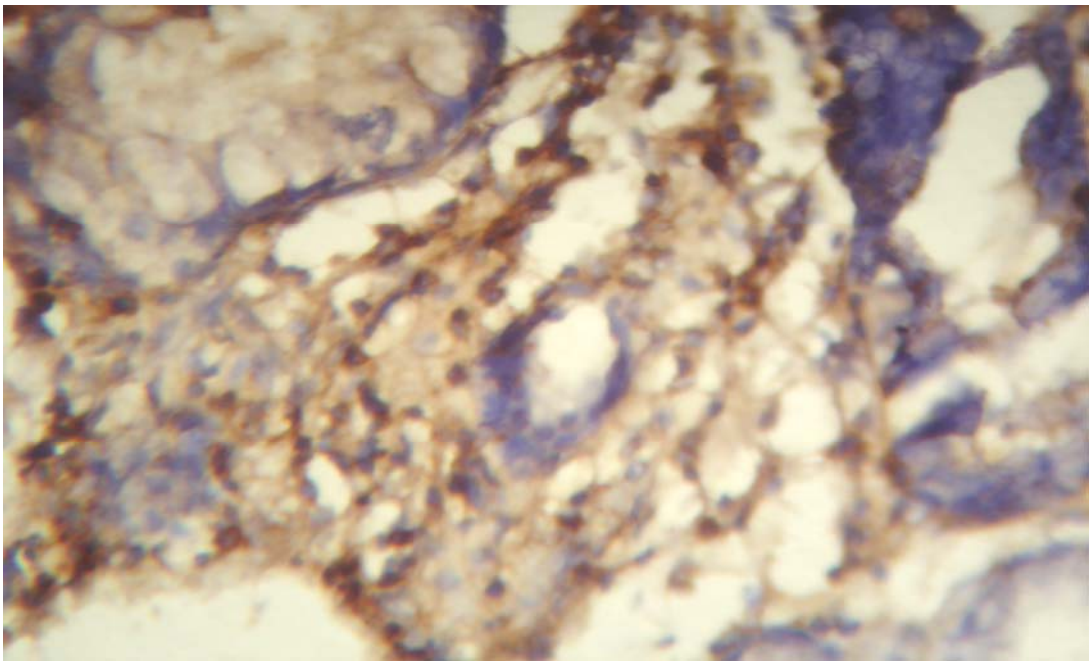


Fig.46: Section shows the same colonic mucosa as in Fig.44 showing CD 20 positive cells without increase in the intraepithelial lymphocytes.

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Sl.No.	BiopsyNo	Name	Age	M/F	Clinical H/O	Endoscopy	CD - 3	CD - 20	CD - 45	CK	HPE	No of lymphocytes/100 epithelial cells
1	23/05	Shyamala	48	F	Diarrhoea	D3 nodules	-	-	-		To consider tropical sprue	5
2	25A/05	Murugan	30	M	Diarrhoea	D2 nodules	-	-	+	-	Non specific Duodenitis	28
3	25B/05	Kumaran	30	M	Diarrhoea	D3 nodules	-	-	+	-	Non specific Duodenitis	24
4	306/05	Hanifa	28	M	Dyspepsia	D2 tiny polyps	-	-	-	-	Non specific Duodenitis	Occassional
5	397/05	Venkatesan	28	M	Diarrhoea	D2 nodules	+	-	-	-	Non specific Duodenitis	15
6	541/05	Rajini	25	M	Pain-abdomen	Polyps-trans colon	+	-	+	-	Ulcerative colitis	23
7	613/05	Ramayee	75	F	? IBD	Nodules-rectum	+	-	+	-	Ulcerative colitis	19
8	839/05	Anjeeran	47	F	Malabsorption	normal	+	+	+	-	Non specific Duodenitis	18
9	1085/05	Komalavalli	42	F	Mucous Diarr	Nodules-rectum	-	-	-	-	Non specific colitis	Occassional
10	1324/05	Ranganathan	46	M	Dyspepsia	Nodules-stomach	-	-	+	-	Chronic gastritis	21
11	1339/05	Sundarbalan	45	M	Dyspepsia	Nod-Asc colon	-	-	+	-	Crohn's disease	26
12	1342/05	Thalappan	52	M	Malabsorption	Irregular D2 mucosa	+	-	+	-	Chronic gastritis	21
13	1807/05	Majith	52	M	Dyspepsia	Antral nodule	-	+	-	+	Follicular gastritis	26
14	1850/05	Devaki	57	F	Diarrhoea	D3 nodules	+	-	-	-	Non specific Duodenitis	12
15	1863/05	Venkatesan	33	M	Dyspepsia	Nodules-stomach	+	-	+	-	Immunoproliferative diseas	38
16	2155/05	Kasthuri	42	F	Diarrhoea	Irregular D2 mucosa	+	-	-	-	Non specific Duodenitis	12
17	2205/05	Saranya	8	F	Mucous Diarr	D3 nodules	+	-	+	-	Non specific Duodenitis	31
18	2828/05	Murugan	24	M	Diarrhoea	Colon congested	-	-	+	-	Non specific colitis	12
19	2948/05	Pushpa	50	F	Dyspepsia	Antral nodule	-	-	-	-	Chronic gastritis	Occassional
20	3241/05	Fathima	50	F	Diarrhoea	D3 nodules	+	+	-	-	Non specific Duodenitis	18
21	3273/05	Chandran	36	M	Diarrhoea	D3 nodules	-	+	+	-	Non specific Duodenitis	26
22	3317/05	Thirupathy	43	M	IBS	Colon congested	+	-	+	-	Non specific colitis	13
23	3317a/05	T pathy	43	M	IBS	Nodules-rectum	-	+	+	-	Non specific colitis	18
24	1172/06	Munusamy	32	M	Dyspepsia	D2 nodules	+	-	+	-	Non specific Duodenitis	12
25	1316/06	Rukmani	42	F	Diarrhoea	Ileum	-	-	+	-	Non specific ileitis	26

26	1651/06	Abdul	65	M	Dyspepsia	Nodules-pylorus	-	-	-	-	Poorly diff. Ca	Occassional	
27	1065/06	Sebastan	58	M	Diarrhoea	D2 nodules	-	+	+	-	Non specific Duodenitis		17
28	2669/06	Sangeeta	35	F	Mucous Diarr	Colon congested	-	-	-	-	Crohns to be considered	Occassional	
29	3623/06	Srikanth	28	M	Diarrhoea	caecum-inflamed	-	-	+	-	Non specific colitis		15
30	4135/05	Vasantha	47	F	Dyspepsia	Thickened gastric mucosa	-	-	-	-	Follicular gastritis	Occassional	
31	853/06	Krishnasamy	85	M	Dyspepsia	caecum-inflamed	-	-	+	-	Non specific colitis		12
32	3364/05	Lakshmi	56	F	Dyspepsia	Antral nodule	-	-	-	-	Chronic gastritis	Occassional	
33	1828/06	Kanagaraj	55	M	Dyspepsia	Antral nodule	-	-	+	-	Indicative of lymphoma		42
34	1032/06	Gurusamy	50	M	Diarrhoea	D3 nodules	-	-	-	-	Non specific Duodenitis	Occassional	
35	3455/05	Mashabooli	48	F	Dyspepsia	Ulcer-pylorus	-	-	-	-	Chronic gastritis	Occassional	
36	3453/05	Kalaiselvi	39	F	Diarrhoea	Colon congested	-	+	+	-	Non specific colitis		18
37	3507/05	Balakrishnan	36	M	GOO	Growth pylorus	-	-	-	+	Poorly diff. Ca	Occassional	
38	1004/06	Muthammal	65	F	Dyspepsia	D2 nodules	-	-	-	-	Non specific Duodenitis	Occasssional	
39	1362/06	Jeeva	44	F	Dyspepsia	D2 nodules	-	-	-	-	Non specific Duodenitis	Occassional	
40	1390/06	Thulasidass	18	M	Diarrhoea	D3 nodules	-	-	-	-	Non specific Duodenitis	Occassional	
41	1392/06	Mumtaz	43	F	Diarrhoea	D3 nodules	-	-	-	-	Non specific Duodenitis	Occassional	
42	1529/06	Radha	65	F	Diarrhoea	D3 nodules	-	-	-	-	Non specific Duodenitis	Occassional	
43	2626/06	Kondiah	40	M	Ileocecal narrow	caecum-inflamed	-	-	+	-	Non specific colitis		24
44	2056/06	Elumalai	28	M	IBD	Inflammed Asc colon	+	-	+	-	Ulcerative colitis		20
45	1818/06	Unnikrishnan	66	M	Dyspepsia	Antral nodule	-	-	-	-	Chronic gastritis	Occassional	
46	1993/06	Kondiah	40	M	Diarrhoea	Colon congested	-	+	-	-	Non specific colitis		15
47	1906/06	Arputhan	38	M	GOO	Growth pylorus	-	-	-	+	Poorly diff. Ca	Occassional	
48	1432/06	Jaheerbegum	44	F	Dyspepsia	D2 nodules	-	-	-	-	Non specific Duodenitis	Occassional	
49	3929/05	Gnanamoorty	53	M	Malena	caecum-inflamed	-	-	-	-	Non specific colitis	Occassional	
50	3783/05	Durairaj	52	M	Dyspepsia	pylorus-unhealthy	-	-	-	-	Chronic gastritis	Occassional	